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(54) Title: METHOD OF INTRODUCING PATHOGEN RESISTANCE IN PLANTS		
(57) Abstract <p>Variegated plants have increased pathogen resistance: cells of the plant express a phenotype, which may comprise necrosis and/or a plant defence response, and other cells not expressing this phenotype have increased pathogen resistance. Embodiments of the invention employ various genes, including <i>Cladosporium fulvum</i> pathogen resistance genes, which are inactivated, for example as a result of insertion of a transposable genetic element, and then reactivated in plant cells to result in necrosis and/or a plant defence response, leading to increased pathogen resistance. Cells, plants and other compositions of matter are provided comprising various combinations of genes involved in this system.</p>		

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METHOD OF INTRODUCING PATHOGEN RESISTANCE IN PLANTS

The present invention relates to a method of introducing pathogen resistance in plants, particularly broad spectrum pathogen resistance, and plants which
5 may be obtained by said method and which show resistance to at least one but preferably more than one pathogen.

Plants are constantly challenged by potentially pathogenic microorganisms. Crop plants are
10 particularly vulnerable, because they are usually grown as genetically uniform monocultures; when disease strikes, losses can be severe. However, most plants are resistant to most plant pathogens. To defend themselves, plants have evolved an array of both
15 preexisting and inducible defences which include barriers to pathogen entry such as thickened or chemically crosslinked cell wall components or toxic chemicals derived from complex plant biosynthetic pathways. Pathogens must specialize to circumvent the
20 defence mechanisms of the host, especially those biotrophic pathogens that derive their nutrition from an intimate association with living plant cells. If the pathogen can cause disease, the interaction is said to be compatible, but if the plant is resistant, the
25 interaction is said to be incompatible.

Induced resistance is strongly correlated with the hypersensitive response (HR), an induced response

associated with localized cell death at sites of attempted pathogen ingress. It is hypothesized that by HR the plant deprives the pathogen of living host cells but there is no certainty about whether localised cell death results from or induces plant defence mechanisms.

Many plant defence mechanisms are strongly induced in response to a challenge by an unsuccessful pathogen. Such an induction of enhanced resistance can be systemic (hereinafter referred to as systemic acquired resistance (SAR)) (Ross, 1961; Ryals et al., 1992). Acquired resistance can also be local (hereinafter referred to as LAR) (Ryals et al., 1992). Acquired resistance has been extensively researched and various facts have been established. For example, biotic stimuli are required to provoke the HR resulting in areas of dead plant cells on the leaf. Cell death resulting from wounding or other abiotic stresses will not suffice. (Ryals et al., 1992; Enyedi et al., 1992). In addition, SAR is correlated with the induction of a large array of pathogenesis-related (PR) proteins, some of which have demonstrated anti-fungal activity (Ward et al., 1991).

A variety of examples of SAR have been studied and include challenging of tobacco carrying the N gene for resistance to tobacco mosaic virus (TMV) with TMV (Ross, 1961) and challenging cucumber seedlings with tobacco necrosis virus or *Colletotrichum largenarium*.

Results show that a challenge with one pathogen leads to enhanced resistance to a wide variety of other pathogens (Ryals et al., 1992).

SAR has also been correlated with increased
5 levels of salicylic acid in plants which have been challenged by pathogens (Malamy et al., 1990; Mettraux et al., 1990) which has been confirmed by studies that show that a supply of exogenous salicylic acid to unchallenged plants can result in SAR (Ward et al.,
10 1991; Hennig et al., 1993). Transgenic plants designed so that salicylic acid accumulation is prevented by expression of a salicylate hydroxylase gene show reduced SAR compared to non-transgenic plants where salicylic acid accumulation is not prevented (Gaffney
15 et al., 1993). SAR can also be induced by many chemicals manufactured by Ciba-Geigy such as 2,6-dichloroisonicotinic acid (INA) (Uknes et al., 1992).

SAR is an attractive method by which broad spectrum disease control can be achieved. However, two
20 major drawbacks hinder its commercial exploitation: SAR is not a heritable trait and so the phenomenon has to be successfully induced into every plant in the crop stand; to be effective throughout the crop's life, the SAR phenotype has to be re-boosted at regular
25 intervals.

Although the mechanisms causing SAR are not fully understood, it is believed that when a plant is

challenged by a pathogen to which it is resistant, it undergoes an HR at the site of attempted ingress of the incompatible pathogen. The induced HR leads to a systemic enhancement and acquisition of plant
5 resistance to virulent pathogens that would normally cause disease in the unchallenged plant.

It has long been known that HR-associated disease resistance is often (though not exclusively) specified by dominant genes (R genes). Flor showed that when
10 pathogens mutate to overcome such R genes, these mutations are recessive. Flor concluded that for an R gene to function, there must also be a corresponding gene in the pathogen, an "avirulence gene" (Avr gene). To become virulent, pathogens must thus stop making a
15 product that activates R gene-dependent defence mechanisms (Flor, 1971). A broadly accepted working hypothesis, often termed the elicitor/receptor model, is that R genes encode products that enable plants to detect the presence of pathogens, provided said
20 pathogens carry the corresponding AVR gene (Gabriel and Rolfe, 1990). This recognition is then transduced into the activation of a defence response.

The mlo allele of the Mlo gene of barley is the one example of a recessive disease resistance gene
25 currently widely used in plant breeding. Lines that are homozygous for the recessive allele of this gene activate the defence response (comprising formation of

cell wall appositions) even in the absence of the pathogen (Wolter et al, 1993). Thus the mlo mutation causes a defence mimic phenotype, also known as a necrotic or disease lesion mimic phenotype, and appears to deregulate the defence response, so that it is activated precociously, or is regulated on more of a "hair trigger". A number of examples of such disease lesion mimic mutants exist in maize (Johal et al, 1994, Pryor, 1987, Walbot, 1983). Recently, such mutants have been sought in Arabidopsis. The characterization of one such mutant, *acd1*, has been reported (Greenberg and Ausubel, 1993). Further mutants of this type have been reported at scientific meetings (the Arabidopsis *acd2* mutation by F.M. Ausubel at a meeting at Rutgers University, New Jersey, USA, April 1993; Arabidopsis mutations now known as *lsd* (for lesions simulating defence response) mutations by R. Dietrich and J. Dangl at the ARAPANET ((Arabidopsis Pathology Network) workshop in Wye College, Kent, UK in April 1993). Manuscripts describing the *acd2* and *lsd* mutations are Dietrich et al. and Greenberg et al. (1994). It is highly likely that the recessive mutations identified in such mutant screens that leave the defence response more constitutively on, or more rapidly activated, or less easily inactivated, are in genes that normally dampen down the defence response to prevent it becoming so severe that it is deleterious to the plant.

Conceivably, such gene could be cloned, expressed in an antisense or sense configuration to reduce expression of the corresponding gene (Hamilton, 1990, Napoli et al, 1989).

5 Pathogen avirulence genes are still poorly understood. Several bacterial Avr genes encode hydrophilic proteins with no homology to other classes of protein, while others carry repeating units whose number can be modified to change the range of plants on
10 which they exhibit avirulence (Keen, 1992; Long and Staskawicz, 1993). Additional bacterial genes (*hrp* genes) are required for bacterial Avr genes to induce HR, and also for pathogenicity (Keen, 1992; Long and Staskawicz, 1993). It is not clear why pathogens make
15 products that enable the plant to detect them. It is widely believed that certain easily discarded Avr genes contribute to but are not required for pathogenicity, whereas other Avr genes are less dispensable (Keen, 1992; Long and Staskawicz, 1993). The characterization
20 of two fungal avirulence genes, Avr 4 and Avr 9 (De Wit et al., 1992; Joosten et al., 1994), has also been reported. Research is also being undertaken to clone rice blast avirulence genes from the causal organism *Magnaporthe grisea* and the avirulence genes (NIP
25 proteins) of the barley pathogen *Rhynchosporium secalis*. Two viral avirulence genes have also previously been cloned. Culver and Dawson, 1991, have

shown that tobacco mosaic virus coat protein is the avirulence determinant for the N' gene product. In addition, the potato virus X coat protein appears to be the avirulence determinant for the Rx and Nx genes
5 (Kavanagh et al., 1992; Santa-Cruz et al., 1993; Köhm et al., 1993; Goulden et al., 1993).

Recently the map based cloning of the tomato *Pto* gene that confers "gene-for-gene" resistance to the bacterial speck pathogen *Pseudomonas syringae* pv tomato
10 (*Pst*) has been reported (Martin et al., 1993). It has also been recently reported that the *Arabidopsis* *Rps2* gene (which confers *Pseudomonas syringae* resistance) and the tobacco N gene (which confers virus resistance) have been cloned (Keystone Symposium, January 1994).
15 Even more recently, the *Rps2* and features of the *Cf-9* gene sequences have been revealed at the 13th Annual Symposium in Columbia, Missouri, April 13th-16th 1994, on the Biology of Communication in Plants.
International Patent Application No: PCT/GB94/02812
20 describes a method for generally identifying and cloning plant resistance genes.

The technology for gene isolation based primarily on genetic criteria has improved dramatically in recent years, and many workers are currently attempting to
25 clone a variety of R genes. Targets include (amongst others) rust resistance genes in maize, *Antirrhinum* and flax (by transposon tagging); downy mildew resistance

- genes in lettuce and *Arabidopsis* (by map based cloning and T-DNA tagging); *Cladosporium fulvum* (Cf) resistance genes in tomato (by tagging, map based cloning and affinity labelling with avirulence gene products);
- 5 virus resistance genes in tomato and tobacco (by map based cloning and tagging); nematode resistance genes in tomato (by map based cloning); and genes for resistance to bacterial pathogens in *Arabidopsis* and tomato (by map based cloning).
- 10 Tomato (*Lycopersicon esculentum*) is susceptible to disease caused by the leaf mould fungal pathogen *Cladosporium fulvum*. According to De Wit, 1992, the Avr9 gene of *C. fulvum*, which confers avirulence on *C. fulvum* races that attempt to attack tomato varieties
- 15 that carry the Cf-9 gene, encodes a secreted cysteine-rich peptide with a final processed size of 28 amino acids. However, its role in compatible interactions is not clear. The R genes (Cf-genes) that act against *C. fulvum* have been identified and bred into cultivated
- 20 varieties, often from related species of tomato (Dickinson et al., 1993; Jones et al., 1993).

It has been shown that *C. fulvum* contains Avr genes that confer recognition by plants which contain the Cf-genes, leading to activation of host defence

25 mechanisms to attack the disease (incompatibility). The Avr4 and Avr9 genes encode small peptides that are secreted by the pathogen into the intercellular spaces

of infected leaves, from which they can be extracted. This has enabled the purification and sequencing of these peptides and the isolation of the genes that encode them (De Wit, 1992; Joosten et al., 1994).

5 Experiments have shown that when the Avr9 gene is transformed into a race of pathogen that lacks Avr9, then the race of pathogen becomes avirulent on plants which are carrying the Cf-9 gene. In addition, it has been shown that disruption of the Avr9 gene in a
10 pathogen race which is avirulent on plants carrying Cf-9 gene confers compatibility on the Cf-9 containing plants (Van Den Ackerveken et al., 1992, Marmeisse et al., 1993).

In addition, De Wit and colleagues have further
15 shown that the secreted peptide encoded by the Avr9 gene can be injected into Cf-9 containing tomato leaves to elicit a necrotic response in the injected area. The necrotic response is consistent with local and vigorous activation of a defence response (De Wit,
20 1992; WO 91/15585). International Patent Application No. PCT/GB94/02812 describes the transgenic expression of the Avr9 gene using the strong cauliflower mosaic virus 35S plant promoter to cause lethality in Cf-9 plants. This transgenic expression has been used to
25 select mutants in which the Cf-9 gene has been inactivated by transposon insertion in order to isolate the Cf-9 gene and perform DNA sequence analysis of this

gene.

Various pathogen races that overcome these *Cf*-genes have emerged and are named after the *Cf*-gene which they can overcome. For example, *C. fulvum* race 4
5 can overcome *Cf*-4; *C. fulvum* race 5 can overcome *Cf*-5 and *C. fulvum* race 2.4.5.9 can overcome *Cf*-2, *Cf*-4, *Cf*-5 and *Cf*-9.

WO 91/15585 describes a hypothetical method whereby if a *Cf*-9 gene and/or an *Avr*9 gene were
10 expressed under the control of a promoter that is induced by a broad range of pathogens, then a general defence response could be induced. However, there is a lack of enabling disclosure regarding which
polynucleotide sequences could be used either as the
15 resistance gene or as an actual promoter which would be suitably affected by a broad range of pathogens. A further problem with this proposed method is that necrosis induced by the *Cf*-9 and *Avr*9 gene combination could lead to further induction of *Avr*9 and/or *Cf*-9
20 leading to spreading of the necrosis and severe reduction in the yield of the plant. This problem may arise since promoters such as promoters for plant defence genes and other genes involved in the defence response such as PR genes (pathogenesis related genes),
25 are induced in both a compatible and an incompatible interaction. Therefore, even if a promoter exists which is effectively induced by a broad range of

pathogens, the method would not be viable unless the promoter is only induced by the appearance of a compatible pathogen. If the defence response provides further induction of the promoter the plant might
5 experience spreading necrosis.

The present invention has resulted from experiments involving transposon tagging of resistance genes, the first one being *Cf-9*. Numerous alleles of the *Cf-9* gene (*Cf-9*Ds*) were isolated that had been
10 inactivated by the maize element *Dissociation (Ds)*. These inactive *Cf-9*Ds* genes did not give rise to a constitutive and lethal activation of defence mechanisms in response to constitutively expressed *Avr9* transgene (*35S:SP:Avr9*). On backcrossing plants that
15 carried the *Cf-9*Ds* and *35S:SP:Avr9* genes to tomato plants carrying an Activator (*Ac*) transposase gene (*sAc*) in the homozygous state but lacking *Cf-9*, a quarter of the resultant progeny carried *sAc*, *35S:SP:Avr9* and *Cf-9*Ds*. These plants showed somatic
20 excision of *Ds* from the *Cf-9*Ds* gene, somatically restoring *Cf-9* function and giving rise to localised activation in cells of plant defence responses due to recognition of the constitutively expressed *Avr-9* peptide. These cells died and gave rise to small
25 necrotic sectors, the plants phenotypically showing variegation for a defence-related necrosis, similar to somatic flecks of necrosis that are associated with the

induction of SAR in plants challenged with necrotising pathogens. Further work showed that plants that variegate for somatic sectors of plant defence response in this way have increased resistance to a range of
5 pathogens.

Thus, a first aspect of the present invention relates to a method of providing pathogen resistance, in particular broad spectrum pathogen resistance, in plants by induction of variegation in which genes are
10 expressed or suppressed resulting in the activation of necrosis. A method according to the present invention comprises: (i) inactivating a nucleotide sequence which contributes to plant cell necrosis or inactivating one or more nucleotide sequences forming part of a
15 combination of nucleotide sequences which contribute to plant cell necrosis; (ii) introducing said nucleotide sequence or sequences into the genome of a plant; and (iii) restoring said nucleotide sequence or sequences to a functional form to yield a level of necrosis
20 resulting in pathogen resistance. The plant cell necrosis is preferably defence-related plant cell necrosis.

A second aspect of the present invention relates to a method of providing pathogen resistance in plants
25 by induction of variegation in which genes are expressed or suppressed resulting in the activation of a plant defence response which comprises: (i)

inactivating a nucleotide sequence which contributes to the plant defence response or inactivating one or more nucleotide sequences forming part of a combination of nucleotide sequences which contribute to the plant
5 defence response; (ii) introducing said nucleotide sequence or sequences into the genome of a plant; and (iii) restoring said inactivated nucleotide sequence or sequences to a functional form to result in pathogen resistance.

10 The variegation will generally be for somatic sectors. Pathogen resistance will generally be increased compared with wild-type.

 The nucleotide sequence or sequences comprise one or more genes. The plant defence response and/or plant
15 cell necrosis occurs on expression of the gene or genes. The defence response and/or plant cell necrosis can be conditional or unconditional on the expression of one or more interacting genes. A substance or a combination of substances may result in increased
20 pathogen resistance. Examples are discussed further below.

 For example, the nucleotide sequence or sequences may comprise a gene encoding either a substance which leads to necrosis; e.g. through activation of the plant
25 defence response, or a substance which leads to a plant defence response with no sign of necrosis. For example, the sequence or sequences may comprise a plant

pathogen resistance gene (R), an avirulence gene (Avr) or other elicitor or ligand gene (L) of an R gene, or both an R gene and an L gene.

The inactivation of the nucleotide sequence or
5 sequences which contribute to the plant defence response and/or plant cell necrosis is preferably effected by insertion of a transposable genetic element into the nucleotide sequence or one or more of the nucleotide sequences forming a combination of
10 nucleotide sequences. The transposable genetic element is preferably a transposon or a nucleotide sequence flanked by specific nucleotide sequences so that transposon excision gives rise to activation of the plant defence response and/or necrosis. Thus,
15 insertion of a genetic lesion into the nucleotide sequence disrupts the gene to prevent expression of a product able to function in contributing to the plant defence response and/or plant cell necrosis. In the absence of the lesion, e.g. following excision of a
20 transposable element such as a transposon, the gene may be expressed to produce a functional product, i.e. gene function is restored. The lesion may be inserted into the part of the gene coding for the expression product, or may be in a regulatory sequence such as a promoter
25 required for expression of the product.

In this form of the invention, re-activation within the plant is preferably carried out by

restoration of the inactivated nucleotide sequence or sequences resulting in activation of a plant defence response and/or necrosis. Such restoration may be caused or allowed by culturing of the plant. Where the nucleotide sequence is inactivated by virtue of insertion of a transposable element therein, the plant genome should contain at least one nucleotide sequence coding for a corresponding transposon activation system (for example, comprising a transposase).

Alternatively, the inactive form could be flanked by recombinase recognition sequences that are acted on by a site specific recombination system (comprising a specific recombinase) so that recombination activates the inactive form of the gene. Hence, when the inactivated nucleotide sequence or sequences are introduced into the plant genome somatic excision of the transposon or recombination of the nucleotide sequence occurs in some cells leading to activation of the plant defence response and/or necrosis in specific clones of cells.

The number of cells in which restoration of function occurs may vary. As discussed further below, certain measures are available for optimising the system, e.g. by controlling the frequency of spontaneous excision of a transposable element which is caused or allowed upon cultivation of a plant with the requisite nucleotide sequence or sequences within its

genome.

The present invention further provides transgenic plants having increased pathogen resistance obtainable by the method of the present invention, and any clone
5 of such a plant, seed, selfed or hybrid progeny and descendants, and any part of any of these, such as cuttings, seed. The invention provides any plant propagule, that is any part which may be used in reproduction or propagation, sexual or asexual,
10 including cuttings, seed and so on. Derivatives of plants are also provided by the present invention. A derivative is any functional unit derived therefrom howsoever achieved (e.g. functional allele of gene made by mutagenesis, recombinant DNA, synthesis, or
15 plant which could not have been produced without the use or manufacture of the plant from which it is derived.)

Transgenic plants in accordance with the present invention may demonstrate increased pathogen resistance
20 since the induced plant defence response and/or necrosis of plant cells may cause other cells, such as adjacent cells, to acquire pathogen resistance. The activation of, for example, a plant resistance gene in a plant cell is inherited by the progeny and
25 descendants of that cell. The expression of this plant resistance gene leads to initiation of the defence response in cells which may eventually lead to the

death of the participating plant cells resulting in an area of plant cell necrosis. So, plants may have variegation for small somatic sectors in which defence-related plant cell necrosis is activated. This response may induce pathogen resistance in other cells. In an alternative, operating on the same general principle, the expression of one or more plant pathogen resistance gene may either lead to initiation of the defence response only resulting in variegation for small somatic sectors in which the plant defence response is activated or of plant cell necrosis which is not related to the plant defence response resulting in variegation for small somatic sectors in which plant cell necrosis is activated.

Hence, the plant may acquire resistance to a broad range of pathogens and not only to the pathogen associated with the gene or genes contributing to necrosis, for example, *C. fulvum* in the case of the *Cf-9/Avr* gene combination. For example, a transgenic tomato plant according to the present invention may demonstrate resistance against a broad range of pathogens such as one or more bacterial plant pathogens (for example, *Xanthomonas campestris*, *Pseudomonas syringae*), fungal plant pathogens (for example, *Phytophthora infestans*, *Fusarium oxysporum*, *Botrytis cinerea*, *Verticillium dahliae*, *Altenaria solani*, *Rhizoctonia solani*) and viral pathogens (for example,

TMV, PVX, PVY, TSWV). Similarly, other transgenic plants such as transgenic tobacco, *Arabidopsis* and potato plants may display resistance to a large number of major diseases of important crop species such as,
5 Peronospora, Phytophthora, Puccinia, Erysiphe and Botrytis.

Thus, according to a further aspect of the invention there is provided a plant, or any part thereof, which is phenotypically variegated, with
10 clones of cells expressing a first phenotype and other cells expressing a second phenotype which is increased pathogen resistance compared with wild-type. The first phenotype is preferably necrosis and/or a plant defence response phenotype. As discussed, plants variegated by
15 somatic sector for such a phenotype may have enhanced pathogen resistance as a result of a second phenotype in cells, which may be adjacent to the cells with the first phenotype which are necrotic and/or in which a plant defence response is activated. The phenotypic
20 variegation is likely to result from expression in cells with the first phenotype of a gene or gene, or nucleic acid comprising a gene or genes, which contributes to such phenotype, whereas other cells without such phenotype lack such gene expression. As
25 discussed herein, this may result from reactivation of a previously inactivated gene, such as a resistance gene, for example by random excision of a transposable

element such as a transposon.

In a further aspect, the present invention provides a host cell, such as a plant or microbial cell, or a plant comprising at least one such cell, containing (i) nucleic acid encoding one or more nucleotide sequences which cause or contribute to the plant defence response and/or cell necrosis, at least one of the nucleotide sequences being reversibly inactivated, for example by insertion of a transposable element such as a transposon, and (ii) nucleic acid encoding a molecule able to reverse the inactivation, such as, in the case of a transposon, a transposase. Thus, the cell may comprise a plant resistance gene or other gene involved in the plant defence response or able to kill a cell when expressed therein (either alone or in combination with one or more sequences, for example in the case of an *R* gene the corresponding elicitor), the gene being inactivated by insertion therein of a transposon, and the cell further comprising a gene encoding a transposase.

In an exemplary embodiment, the genome of the cell comprises the gene *Cf-9*, or a mutant, derivative, variant or allele thereof which retains *Cf-9* function, inactivated by insertion therein of a transposon, the genome also comprising the *Avr-9* gene, or a mutant, derivative, variant or allele thereof which retains *Avr-9* function, and a gene encoding a transposase able

to excise the transposon from the *Cf-9* gene or functional equivalent. Other resistance genes may be employed, as may genes which do not require the presence of an elicitor molecule to cause cell
5 necrosis, as discussed further elsewhere herein.

The cell may comprise the nucleic acid encoding the various genes by virtue of introduction into the cell or an ancestor thereof of the nucleic acid, e.g. by transformation, using any suitable technique
10 available to those skilled in the art. Furthermore, plants which comprise such cells, and seed therefore, may be produced by crossing suitable parents to create a hybrid whose genome contains the required nucleic acid, in accordance with any available plant breeding
15 technique. For example, a parent strain comprising within its genome a plant resistance gene containing a transposon or other inactivating lesion may be crossed with a second strain comprising within its genome a gene encoding the elicitor molecule for the plant
20 resistance gene and a suitable transposase for excision of the transposon. At least a proportion of the hybrid progeny of the parents, i.e. seed or plants grown therefrom, will comprise the required nucleic acid for activation in the plant of, in this example, the plant
25 resistance gene and, following interaction with the elicitor, the plant defence response and/or plant cell necrosis.

Plants according to this aspect of the present invention will be variegated genetically. Clones of cells will have one or more nucleotide sequences which cause or contribute to the plant defence response and/or cell necrosis reactivated by removal of the inactivating lesion such as a transposon, so that a first phenotype such as necrosis is shown, while in other cells the sequence or sequences will remain inactivated so these cells will not show the first phenotype.

Within the cell or cells, the nucleic acid may be incorporated within the chromosome. A gene stably incorporated into the genome of a plant is passed from generation to generation to descendants of the plant, so such descendants should show the desired phenotypic variegation and so may have enhanced pathogen resistance.

In addition to a plant, the present invention provides any clone of such a plant, seed, selfed or hybrid progeny and descendants, and any part of any of these, such as cuttings, seed. The invention provides any plant propagule, that is any part which may be used in reproduction or propagation, sexual or asexual, including cuttings, seed and so on.

A further aspect of the present invention provides a method of making such a cell involving introduction of nucleic acid (e.g. a vector) comprising

(i) nucleic acid encoding one or more nucleotide sequences which cause or contribute to the plant defence response and/or cell necrosis, at least one of the nucleotide sequences being reversibly inactivated, for example by insertion of a transposable element such as a transposon, and/or (ii) nucleic acid encoding a molecule able to reverse the inactivation, such as, in the case of a transposon, a transposase into a plant cell. Introduction of nucleic acid (i) may be accompanied, preceded or followed by introduction of nucleic acid (ii). Such introduction may be followed by recombination between the nucleic acid and the plant cell genome to introduce the sequence of nucleotides into the genome. Descendants of cells into which nucleic acid has been introduced are included within the scope of the present invention.

The level of the plant defence response and/or plant cell necrosis in the small somatic sectors should be sufficient to result in the induction of acquired resistance or the induction of other defence mechanisms. Since this method leads to activation of acquired resistance but is inherited it is referred to as Genetic Acquired Resistance (GAR). Hence, any system which gives rise to a variegation leading to GAR is applicable to the present invention.

Methods and plants etc. according to the present invention are particularly beneficial since the

nucleotide sequence or sequences which contribute to the plant defence response and/or plant cell necrosis, for example the avirulence and plant resistance genes, may be under control of any suitable promoter, such as
5 a constitutive promoter or, in the case of R genes, their own endogenous promoter, or a cell type specific promoter. Furthermore, the restoration of the nucleotide sequence or sequences, for example by the somatic excision of a transposon, gives rise to
10 recurrent and widespread induction of the plant defence response in many small clones of cells throughout the plant, irrespective of whether or not there has been a challenge by pathogen. The resistance conferred on the plant is therefore constitutive and broad.

15 The present invention may be used for many applications and is suitable for deployment in F1 hybrid seed production system. In such a system, one of the parents should be homozygous, for example, for the transposase or recombinase gene. In addition, in a
20 system where two components are required for inducing the necrosis such as in the Avr9/Cf-9 gene combination for example, this parent should also be homozygous for the constitutively expressed genes. The other parent should be homozygous for the gene that encodes the non-
25 autonomous inactivation system, such as the transposon or recombinase-recognition sequences. After making a cross between parents of this genetic constitution, on

somatic excision or recombination, the function of the gene or genes which give rise to the defence response and/or plant cell necrosis is restored in somatic sectors in the resulting progeny.

5 It will be clear to the person skilled in the art that any gene or combination of genes which contributes to variegation for the plant defence response and/or plant cell necrosis may be used in the method of the present invention. Furthermore, any system which gives
10 rise to inactivation of the nucleotide sequence or sequences and subsequent restoration of functional sequence or sequences may be used.

 The present invention also provides in further aspects various compositions of matter comprising
15 combinations of nucleotide sequences encoding various substances employed herein. Such combinations of nucleotide sequences which may be introduced into cells in accordance with the present invention follow:

(X) : represents a nucleotide sequence with one
20 or more genes of type X

(XY) : represents a nucleotide sequence with one
 or more genes of type X and one ore more
 genes of type Y etc.

R: receptor gene

25 L: ligand gene (capable of interacting with the R
 gene)

25

I: genetic insert

A: activator of transposition of genetic insert.

R may encode a substance whose presence in a plant results in a plant defence response, necrosis
5 and/or increased pathogen resistance, with I being a genetic insert able to inactivate R and A encoding a substance able to reactivate R inactivated by I:

(1) Any combination of:

1. (R), (I) and (A);
- 10 2. (R) and (IA);
3. (I) and (AR); or
4. (A) and (RI);
5. (RIA).

Alternatively, R and L may encode substances
15 whose presence together in a plant results in a plant defence response, necrosis and/or increased pathogen resistance, I being a genetic insert able to inactivate R and/or L and A encoding a substance able to reactivate R and/or L inactivated by I:

20 (2) Any combination of:

1. (R), (L), (I) and (A);
2. (R), (LI) and (A)
3. (R), (LA) and (I)
4. (R), (IA) and (L)
- 25 5. (L), (IR) and (A)

26

6. (L), (AR) and (I)
7. (I), (LR) and (A)
8. (R) and (LIA)
9. (L) and (IAR)
- 5 10. (I), and (ARL); or
11. (A) and (RLI);
12. (RLIA)

If genetic insert (I) is coupled with either the R or the L gene, the number of possible combinations
10 will then be

(1): (RI) and (A); or
(RIA)

(2): (RI)(L) and (A)
(R), (LI) and (A)
15 (RI) and (LA)
(RA) and (LI)
(RLIA)

Also provided by the present invention is a method of producing a plant, or a part, propagule,
20 derivative or descendant thereof, containing nucleic acid comprising a nucleotide sequence or nucleotide sequences encoding R, I and A, wherein R encodes a substance whose presence in a plant results in a plant

defence response, necrosis and/or increased pathogen resistance, I is a genetic insert able to inactivate R and A encodes a substance able to reactivate R inactivated by I, comprising crossing plant lines whose
5 genomes comprise any of R, I, A and combinations thereof, to produce the plant or an ancestor thereof.

A further aspect provides a method of producing a plant, or a part, propagule, derivative or descendant thereof, containing nucleic acid comprising a
10 nucleotide sequence or nucleotide sequences encoding R, L, I and A, wherein R and L encode substances whose presence together in a plant results in a plant defence response, necrosis and/or increased pathogen resistance, I is a genetic insert able to inactivate R
15 and/or L and A encodes a substance able to reactivate R and/or L inactivated by I, comprising crossing plant lines whose genomes comprise any of R, L, I, A and combinations thereof, to produce the plant or an ancestor thereof.

20 Said plant lines may contain nucleic acid comprising any of R, L, I, A and combinations thereof as a result of transformation of cells of the plant or an ancestor thereof

Herein, unless context demands otherwise, a
25 "receptor" is a product encoded by a gene capable of interacting with another product, the ligand.

Various embodiments of the present invention are

now described in more detail below, by way of example and not limitation.

Nucleotide Sequence or Sequences contributing to the Plant Defence Response and/or Necrosis

- 5 The nucleotide sequence or combination of nucleotide sequences in which at least one of the sequences is inactivated are numerous and may include an engineered allele of a ubiquitin conjugating enzyme (Becker et al., 1993), the CaMV gene VI protein
- 10 (Takashashi et al., 1989), a viral coat protein in the presence of the appropriate viral resistance gene, for example Tobacco Mosaic Virus Elicitor Coat Protein and the gene N' (Culver and Dawson, 1991), a bacterial harpin protein (Wei et al., 1992; He et al., 1993), the
- 15 gene N (see e.g. Whitham et al (1994) and a ToMV-Ob gene cloned by Padgett and Beachy (1993), the potato virus X coat protein and its avirulence determinant, (Kavanagh et al., 1992; Santa-Cruz et al., 1993; Köhm et al., 1993; Goulden et al., 1993), Pto and avrPto
- 20 (see e.g. Rommens et al., 1995), RPS2 of *Arabidopsis thaliana* and the avirulence gene avrRpt2 (Bent et al., Mindrinos et al.), and genes of *Arabidopsis* such as those identified by Greenberg et al. (1994), Dietrich et al., (1994) and Bowling et al., (1994).
- 25 Genes coding for substances leading to rapid cell death, such as BARNASE (Mariani et al., 1990) or

diphtheria toxin (Thorsness et al., 1993) may be usable to induce the changes that lead to GAR even though cell death in these latter examples is not caused by activation of the defence response. It is widely
5 believed amongst researchers in this field that cell death arises from local induction of the defence response and that this cell death can activate adjacent cells to give rise to the defence response. However, the precise cause and effect relationship between these
10 events is not clear at the present time. It is also not clear whether the defence response in plants is necessarily coupled to necrosis. Hence, cells may respond to for example the BARNASE-induced death of adjacent cells by activating a wound-inducible defence
15 response, such as that leading to the activation of protease inhibitors or alkaloid biosynthesis (Ryan 1990). Other genes which may be employed in this way include a proton pump such as a bacterial proton pump like the one expressed by Mittler et al (1995) in
20 transgenic tobacco plants.

A preferred example of the present invention is the use of the *Cf-9/Avr9* gene system. This can involve the matching of a transposon inactivated allele of the *Cf-9* gene to constitutive expression of the *Avr9* gene.
25 This system can be replaced by similar combinations of related genes for example the *Avr4* and *Cf-4* gene, sequence provided herein (cloning of *Cf-4* is described

in a co-pending GB application filed simultaneously with the present application); the *Avr2* and the *Cf-2* gene, sequence provided herein (cloning of *Cf-2* is described in GB 9506658.5, priority from which is claimed herein); the *Avr5* and the *Cf-5* gene, or by cloning resistance genes and corresponding avirulence genes from other systems, such as *RPP5*, sequence provided herein (cloning of *RPP5* is described in GB 9507232.8, priority from which is claimed herein). It certain cases it may be possible to provoke a suitable response in plant cells expressing an R gene in the absence of corresponding *Avr*, for instance by overexpression.

It should also be noted that complete *Avr* or other elicitor gene may not be required. Instead a fragment may be employed, representing a part of the elicitor molecule which interacts to provoke a plant defence response and/or plant cell necrosis.

It is possible that the nucleotide sequence comprises the inactivated R gene, the inactivated *Avr* gene or both, or comprises both the R and *Avr* gene wherein one of the genes is inactivated. Depending of the genes used, the plant defence response and/or plant cell necrosis may be dependent on the expression of both genes and so one example would be that the R gene could be constitutively expressed and the *Avr* gene could exhibit somatic variegation for expression due to

somatic excision and restoration of Avr9 gene expression, or vice versa.

Nucleotide sequences employed in the present invention may encode a wild-type sequence (e.g. gene) selected from those available, or a mutant, derivative, variant or allele, by way of insertion, addition, deletion or substitution of one or more nucleotides, of such a sequence. An alteration to or difference in a nucleotide sequence may or may not be reflected in a change in encoded amino acid sequence, depending on the degeneracy of the genetic code. Preferred mutants, derivatives and alleles are those which retain a functional characteristic of the protein encoded by the wild-type gene, in the present context the ability to contribute to a plant defence response and/or plant cell necrosis. Of course, changes to the nucleic acid which make no difference to the encoded amino acid sequence are included.

Similarly, homologues of the various genes whose use is disclosed herein from other species or races may be employed, as may mutants, variants and derivatives of such homologues.

Inactivation and Reactivation of the nucleotide Sequence or Sequences Contributing to the Plant Defence Response and/or Necrosis

A method according to the present invention may

employ any of a variety of transposon systems known to the skilled person, including the maize Activator/Dissociation (hereinafter referred to *Ac/Ds* system) (Fedoroff, 1989); the maize Enhancer/Suppressor mutator (En/Spm) system (Fedoroff, 1989); and the Antirrhinum Tam1 and Tam3 systems (Coen et al., 1989). In addition, any modified recombination systems which are engineered to yield the appropriate results may be employed, such as, the bacterial Cre-Loxp (Odell et al, 10 1990) or the "FLP/FRT" system (Lloyd and Davis, 1994).

It will be apparent to the skilled person that the particular choice of transposon, recombination or other system used to inactivate the nucleotide sequence or sequences which encode substances leading to the plant defence response and/or plant cell necrosis is 15 not essential to or a limitation of the present invention.

In some systems, a transposon or recombination system might be so active that an unacceptable level of necrosis is seen. If encountered, this may be overcome 20 by engineering alleles of the transposon or recombinase recognition sequence in which the frequency at which activated nucleotide sequences arise is reduced, such as with *Ac(Cla)* (Keller et al., 1993). Alternatively, 25 chemical or site-directed mutagenesis may be used to recover alleles of the necrosis-inducing genes which are less active and therefore result in less severe

levels of plant cell necrosis (Hammond-Kosack et al., 1994).

In other systems, transposition or recombination may be inefficient resulting in too few activated
5 nucleotide sequences leading to an insufficient level of plant cell necrosis. This may be overcome by constructing suitable promoter fusions to the transposase or recombinase gene in the plant gene (Swinburne et al., 1992) to increase the frequency of
10 excision or recombination to efficient levels. The most suitable promoter might give rise predominantly to late small sectors of necrosis during organ development rather than early large sectors.

Many other variations are possible as mechanisms
15 for activating the defence response and/or necrosis after transposon excision or recombination. A form of the *Cf-9* gene may be constructed so that it activates the defence response even in the absence of its ligand. For example, the *Drosophila* receptor *sevenless*
20 (involved in eye development) can be mutated so that it is activated in the absence of its ligand (Basler et al, 1991). For example, high level expression of a disease resistance gene, or expression of a disease resistance gene in another species, may lead to
25 activation of the defence response and/or necrosis even in the absence of an avirulence product. Bonneus, et al (1995). In an alternative, the original disease

resistance gene may be mutated so that it binds to a defined chemical such as an agrichemical and this chemical activates Cf-9 to initiate the defence response and/or necrosis. Hence, genotypic variegation
5 for excision activating the gene may occur, without initiation of the somatic necrotic reaction due to the defence response. The defence response would be initiated when the agrichemical is applied and recognised by the resistance gene triggering the same
10 reaction as if the avirulence gene product were present.

Introducing the Nucleotide Sequence or Sequences which Contribute to Variegation for the Plant Defence Response and/or Necrosis into the Plant Genome

15 The inactivated nucleotide sequence, or combination of nucleotide sequences at least one of which is inactivated, codes for a substance or substances which when expressed in the plant activates the defence response and/or leads to plant cell
20 necrosis resulting in broad spectrum pathogen resistance.

The nucleic acid may be in the form of a recombinant vector, for example a plasmid or agrobacterium binary vector (Van den Elzen et al.,
25 1985). The nucleic acid may be under the control of an appropriate promoter and regulatory elements for

expression in a plant cell. In the case of genomic DNA, this may contain its own promoter and regulatory elements and in the case of cDNA this may be under the control of an appropriate promoter and regulatory elements for expression in the host cell.

Those skilled in the art are well able to construct vectors and design protocols for recombinant gene expression. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

When introducing a chosen gene or gene construct into a cell, certain considerations must be taken into account, well known to those skilled in the art. The

nucleic acid to be inserted may be assembled within a construct which contains effective regulatory elements which will drive transcription. There must be available a method of transporting the construct into the cell.

5 Once the construct is within the cell membrane, integration into the endogenous chromosomal material may or may not occur according to different embodiments of the invention. In a preferred embodiment, the nucleic acid of the invention is integrated into the

10 genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques. Finally, as far as plants are concerned the target cell type should be such that

15 cells can be regenerated into whole plants.

Plants transformed with a DNA segment containing pre-sequence may be produced by standard techniques which are already known for the genetic manipulation of plants. DNA can be transformed into plant cells using

20 any suitable technology, such as a disarmed Ti-plasmid vector carried by *Agrobacterium* exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792, EP-A-444882, EP-A-434616)

25 microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966), electroporation (EP 290395, WO 8706614) or other forms of direct DNA uptake (DE 4005152, WO

9012096, US 4684611). Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. Although Agrobacterium has been reported to be able to transform foreign DNA into some
5 monocotyledonous species (WO 92/14828), microprojectile bombardment, electroporation and direct DNA uptake are preferred where Agrobacterium is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of
10 the transformation process, eg. bombardment with Agrobacterium coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with Agrobacterium (EP-A-486233).

The particular choice of a transformation
15 technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the
20 particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention.

Selectable genetic markers may be used consisting of chimaeric genes that confer selectable phenotypes
25 such as resistance to antibiotics such as kanamycin, hygromycin, phosphinotricin, chlorsulfuron, methotrexate, gentamycin, spectinomycin, imidazolinones

and glyphosate (Herrera-Estrella et al, 1983; van den Elzen et al, 1985).

The present invention is particularly beneficial for use in crop and amenity plants. Examples of
5 suitable plants include tobacco, potato, pepper, cucurbits, carrot, vegetable brassicas, lettuce, strawberry, oil seed brassicas, sugar beet, wheat, barley, maize, rice, soybeans, peas, sunflower, carnation, chrysanthemum, other ornamental plants, turf
10 grass, poplar, eucalyptus and pine.

Still further details of embodiments of the present invention are described in the following non-limiting examples, with reference to the accompanying drawings. In the drawings:

15 Figure 1 schematically depicts the *Cf-9* gene, showing tagged alleles. X marks a probable promoter.

Figure 2 illustrates genetic acquired resistance to *C. fulvum* induced following necrotic sector formation caused by the excision of a Ds element from
20 the *Cf-9* resistance gene in an Avr9 expressing tomato plant. The number of *C. fulvum* pustules per leaf is indicated, 14 days after inoculation.

Figure 3 illustrates genetic acquired resistance to *Phytophthora infestans* (late blight of tomato and
25 potato). GAR+ and GAR- plants from *Cf-9**Ds, mutant

lines M31 and M50 and Cf0 plants spray inoculated with 10,000 sporangiospores/mL. In panel A the appearance of leaves from the mutant 50 experiment 7 days after inoculation is shown. In panel B the rate of leaf
5 abscission (in days after inoculation) in the various genotypes inoculated is given.

Figure 4 illustrates genetic acquired resistance to *Phytophthora infestans* (late blight of tomato and potato). GAR+ and GAR- plants from Cf-9*Ds, mutant
10 lines M31 and M50 and Cf0 plants were spray inoculated with 100 sporangiospores/mL. In panel A the appearance of leaves from the mutant 50 (GAR+ - right-hand) experiment 7 days after inoculation is shown, compared with GAR- (left-hand). In panel B the rate of
15 sporulating lesion formation on the various plant genotypes inoculated is given, with the mean number of sporulating lesions/leaflet given at 5, 7, 10, 13 and 16 days after inoculation.

Figure 5 shows genetic acquired resistance to
20 *Oidium lycopersici* (powdery mildew disease). GAR+ and GAR- plants from Cf-9*Ds, mutant lines M31 and M50 and Cf0 plants were painted with equivalent numbers of spores. In panel A the appearance of leaves 14 days after inoculation is shown, GAR- on the left, GAR+ on
25 the right. In B, the rate of chlorotic lesion (upper panel) and sporulating lesion (lower panel) formation on the various plant genotypes is given for Mutant 31:

mean number of lesions given at 7, 10, 14, 21, 24 and 30 days after inoculation. C shows equivalent results for Mutant 50.

Figure 6 shows the appearance of tomato fruits on GAR⁺ (*sAc*, *Cf-9*Ds* - right-hand) and GAR⁻ (*sAc*, *Cf-9*Ds*, *Avr-9* - left-hand) plants from mutant line M23 at 2, 3, 4, 5, 6 and 7 weeks after flower pollination. Dark green sectors formed on the GAR⁺ but not GAR⁻ fruits by 5 weeks. These dark green sectors were not visible on the red fruit.

Figure 7 shows levels of defence-related gene expression in GAR⁺ and GAR⁻ plants from *Cf-9*Ds* mutant lines M23, M31 and M50 just prior to the pathogen inoculation experiments. Northern analysis shows in panel A the levels of a basic β -1,3 glucanase gene transcript and in panel B the levels of an anionic peroxidase gene transcript.

Figure 8 illustrates functional expression of the *Cf-9* gene under the control of its own promoter in tobacco and potato. In panel A is shown a tobacco leaf that has been injected with intercellular fluid (IF) either containing the Avr9 peptide or lacking the Avr9 peptide. Avr9⁺ IF was obtained from transgenic tobacco or a compatible *C. fulvum* - tomato interaction involving race 5. Avr9⁻ IF was obtained from untransformed tobacco or a compatible *C. fulvum* - tomato interaction involving race 2,4,5,9. Grey

necrosis was visible 3-4 h after injection only in the leaf panels that had received the Avr+ IF. In panel B four separate potato leaves are shown that have each been injected with a single type of IF. Only the two
5 leaves that received the Avr9+IF developed grey necrosis by 24 h.

Figure 9 shows development of the necrotic lethal phenotype in seedlings from the tobacco cross cv. Petite Havana 6201A (35S:SP:Avr9)homozygote x cos 34.1
10 (genomic Cf-9) heterozygote. A time course for the period 5-12 days after seed planting (dsp) is shown. 50% of the seedlings become chlorotic and die within 2 days of seed germination.

Figure 10 shows development of the necrotic
15 lethal phenotype in seedlings from the *Arabidopsis* cross 6201B4 (35S:SP:Avr9)heterozygote x cos 138 (genomic Cf-9) heterozygote. Appearance of seedlings 19 days after the majority of seedlings had germinated. One seedling has died and another has necrotic
20 cotyledons.

Figure 11 shows a single T-DNA construct systems to apply GAR to potato plants. The T-DNA contains a Cf-9 gene sequence under the control of its own promoter which has been inactivated by an autonomous Ac
25 element that is only capable of a low level of excision, the Ac (Cla) element (Keller et al. 1993; Schofield et al. 1994) and the 35S:SP:Avr9 transgene.

Figure 12 shows a photograph of three leaves, two of which are diseased with *C. fulvum* and one which is expressing GAR and is resistant to the same inoculum of *C. fulvum*.

5 Figure 13 illustrates how GAR⁺ plants may be made by crossing stable lines (1) comprising a *Cf-9* gene, inactivated by insertion of a *Ds* transposon, and an *Avr-9* gene and (2) an *Ac* transposase gene, as described in Example 1.

10 Figure 14 illustrates basic simplified haploid crossing schemes to produce plants with increased disease resistance.

T: transgenic line

P: offspring of transgenic line

15 T₁/P₁: line comprising in its genome at least one of each of the four genes, R, L, I or A

T_{1,2}/P_{1,2} line comprising in its genome at least one of each of two of the four genes R, L, I or A

20 T₃/P₃: line comprising in its genome at least one of each of the four genes R, L, I or A not present in T_{1,2}

25 T_{3,4}/P_{3,4}: line comprising in its genome at least one of two of the four genes R, L, I or A not present in T_{1,2}

T_{1,2,3}/P_{1,2,3}: line comprising in its genome at

least one of each of three of the
four genes R,L,I or A
T₄/P₄ line comprising in its genome at
least one of each of the four genes
5 R,L,I or A not present in T_{1,2,3}

SEQ ID NO. 1 shows the genomic DNA sequence of
the *Cf-9* gene. Features: Nucleic acid sequence -
Translation start at nucleotide 898; translation stop
at nucleotide 3487; polyadenylation signal (AATAAA) at
10 nucleotide 3703-3708; polyadenylation site at
nucleotide 3823; a 115 bp intron in the 3' non-coding
sequence from nucleotide 3507/9 to nucleotide 3622/4.
Predicted Protein Sequence - primary translation
product 863 amino acids; signal peptide sequence amino
15 acids 1-23; mature peptide amino acids 24-863.

SEQ ID NO. 2 shows *Cf-9* protein amino acid
sequence.

SEQ ID NO. 3 shows the sequence of one of the *Cf-9*
cDNA clones. Translation initiates at the ATG at
20 position +58.*Cf-9* genomic sequence

SEQ ID NO. 4 shows the amino acid sequence and
DNA sequence of the preferred form of the chimaeric
Avr9 gene used as described herein.

SEQ ID NO. 5 shows the genomic DNA sequence of
25 the *Cf-2.1* gene. Features: Nucleic acid sequence -
Translation start at nucleotide 1677; translation stop

at nucleotide 5012; no consensus polyadenylation signal (AATAAA) exists in the characterised sequence downstream of the translation stop. Predicted Protein Sequence - primary translation product 1112 amino acids; signal peptide sequence amino acids 1-26; mature peptide amino acids 27-1112.

SEQ ID NO. 6 shows *Cf-2* protein amino acid sequence, designated *Cf-2.1*.

SEQ ID NO. 7 shows the amino acid sequence encoded by the *Cf-2.2* gene. Amino acids which differ between the two *Cf-2* genes are underlined.

SEQ ID NO. 8 shows the sequence of an almost full length cDNA clone which corresponds to the *Cf2-2* gene.

SEQ ID NO. 9 shows the genomic DNA sequence of the *RPP5* gene. Anticipated introns are shown in non-capitalised letters. Features: Nucleic acid sequence - Translation start at nucleotide 966; translation stop at nucleotide 5512.

SEQ ID NO. 10 shows predicted *RPP5* protein amino acid sequence.

SEQ ID NO. 11 shows genomic DNA sequence of *Cf-4*. Features of this sequence include: translation start site at nucleotide 201, translation stop beginning at nucleotide 2619, consensus polyadenylation sequence beginning at nucleotide 2835, splice donor sequence in 3' untranslated sequence at 2641, splice acceptor sequence ending at nucleotide 2755, proposed site of

polyadenylation at nucleotide 2955.

SEQ ID NO. 12 shows the predicted Cf-4 amino acid sequence. The predicted protein sequence is composed of a primary translation product of 806 amino acids, signal peptide sequence amino acids 1-23, mature peptide amino acids 24-806.

SEQ ID NO. 13 shows double-stranded nucleic acid and deduced amino acid sequence of a ClaI/SalI DNA fragment encoding the PR1a signal peptide sequence fused to a sequence proposed to encode the mature processed form of *C. fulvum* AVR4. Translation initiation codon at nucleotide 5, termination codon beginning at nucleotide 413. Amino acids 1-30 represent the signal peptide and amino acids 31-136 the mature AVR4 peptide.

EXAMPLE 1

GENETIC ACQUIRED RESISTANCE (GAR) USING Cf-9

(i) *Establishing a stock from which gametes carrying a mutagenised Cf-9 gene may be obtained and identified*

During experiments to isolate the Cf-9 gene by transposon tagging, alleles of the Cf-9 gene (Cf-9*Ds) were isolated that had been inactivated by insertion of the transposon Ds (See International Patent Application No. PCT/GB94/02812 for further details). This inactivated Cf-9*Ds gene did not give rise to a

constitutive and lethal activation of defence mechanisms in response to the constitutively expressed 35S:SP:Avr9 gene.

5 We have established the capacity to carry out transposon tagging in tomato using the maize transposon Activator (Ac) and its Dissociation (*Ds*) derivatives (Scofield et al 1992; Thomas et al 1993; Carroll et al 1993). The strategy is founded on the fact that these
10 transposons preferentially transpose to linked sites. Various lines that carry *Dss* at positions are useful, including FT33 (Rommens et al 1992), carrying a *Ds* linked to *Cf-9*, and lines that carry a construct SLJ10512 (Scofield et al 1992) which contains (a) a
15 beta-glucuronidase (GUS) gene (Jefferson et al 1987) to monitor T-DNA segregation and (b) stable Ac (*sAc*) that expresses transposase and can trans-activate a *Ds*, but which will not transpose (Scofield et al 1992).

 The line FT33 did not carry a *Cf-9* gene. We had
20 to obtain recombinants that placed *Cf-9* in cis with the T-DNA in FT33 in order to carry out linked targeted tagging. Two strategies were pursued simultaneously:

 (a) FT33 was crossed to *Cf9*, a stock that carries the *Cf-9* gene. The resulting F1 was then back crossed
25 to *Cf0* (a stock that carries no *Cf-* genes). Progeny that carry the FT33 T-DNA are kanamycin resistant. Kanamycin resistant progeny were tested for the

presence of *Cf-9*; 5 *C. fulvum* resistant individuals were obtained among 180. We also generated progeny that were homozygous for *Cf-9* and carried that *sAc* T-DNA of SLJ10512. These were crossed to the recombinants in which *Cf-9* and FT33 were *in cis*. In the FT33 T-DNA, a transposable *Ds* element is cloned into a hygromycin resistance gene, preventing its function. The somatic transactivation of this *Ds* element, which only occurs in the presence of transposase gene expression, results in activation of the hygromycin resistance. Thus from crossing the recombinants between *Cf-9* and FT33, to the *sAc*-carrying *Cf-9* homozygotes, hygromycin resistant individuals could be obtained which carry *sAc* and FT33, and are likely to be homozygous for *Cf-9*. 140 individuals of this genotype were thus obtained.

(b) To accelerate obtaining individuals that carried *sAc*, FT33, and were *Cf-9* homozygotes, the FT33/*Cf-9* F1 was crossed to a line that was heterozygous for *Cf-9* and *sAc*. 25% of the resulting progeny carried both T-DNAs and were hygromycin resistant, and of those, slightly more than 50% were disease resistant because they carried at least one copy of the *Cf-9* gene. An RFLP marker was available, designated CP46, that enabled us to distinguish between homozygotes and heterozygotes for the *Cf-9* gene (Balint-kurti et al 1993). In this manner two individuals that were *Cf-9* homozygotes, and that

carried both the FT33 T-DNA and sAc, were obtained. These two individuals were multiplied by taking cuttings so that more crosses could be made onto this genotype.

5 (ii) *Establishing a tomato stock that expresses functional mature AVR9 protein*

A likely frequency for obtaining any desired mutation in a gene tagging experiment is less than 1 in 1000, and often less than 1 in 10,000 (Döring, 1989).

10 To avoid screening many thousands of plants for mutations to disease sensitivity, we established a selection for such mutations based on expressing the fungal Avr9 gene in plants.

The sequence of the 28 amino acids of the mature
15 Avr9 protein is known (van Kan et al 1991). It is a secreted protein and can be extracted from intercellular fluid of leaves infected with Avr9-carrying races of *C. fulvum*. For secretion from plant cells, we designed oligonucleotides to assemble a gene
20 that carried a 30 amino acid plant signal peptide, from the Pr1a gene (Cornelissen et al 1987) preceding the first amino acid of the mature Avr9 protein (see SEQ ID NO. 4). The preferred Avr9 gene sequence depicted in
25 Pr-1a signal peptide sequence (Cornelissen et al, 1987) and the Avr9 gene sequence (van Kan et al, 1991). This

reading frame was fused to the 355 promoter of cauliflower mosaic virus (Odell et al 1984), and the 3' terminator sequences of the octopine synthase gene (DeGreve et al 1983), and introduced into binary
5 plasmid vectors for plant transformation, using techniques well known to those skilled in the art, and readily available plasmids (Jones et al 1992). We obtained transformed Cf0 tomato lines that expressed this gene.

10 *(iii) Crossing AVR9 expressing stock with Cf-9 expressing stock*

The transformed lines obtained in (ii) were crossed to plants that carried the Cf-9 gene. When the resulting progeny were germinated, 50% exhibited a
15 necrotic phenotype, that culminated in seedling death. This outcome was only observed in seedlings that contained the Avr9 gene. When the same transformants were crossed to Cf0 plants, the resulting progeny were all fully viable.

20 From selfing the primary transformants, individuals were identified that were homozygous for the Avr9 transgene. When Avr9 homozygotes were crossed to Cf-9, all progeny died. This system thus provides a powerful selection for individuals that carry mutations
25 in the Cf-9 gene.

(iv) *Tagging and inactivating Cf-9*

Individuals that were homozygous for the *Avr9* gene (section (iv)) were used as male parents to pollinate individuals that were homozygous for *Cf-9*,
5 and carried both *sAc* and the *Ds* in the FT33 T-DNA (section (iiia) and (iiib)). Many thousands of progeny resulting from such a cross were germinated. Most died, but some survived.

DNA was obtained from survivors and subjected to
10 Southern blot analysis using a *Ds* probe. It was observed that several independent mutations were correlated with insertions of the *Ds* into a *BglII* fragment of a consistent size. This suggested that several independent mutations were a consequence of
15 insertion of the *Ds* into the same DNA fragment. Using primers to the *Ds* sequence, DNA adjacent to the *Ds* in transposed *Ds*-carrying mutant #18 was amplified using inverse PCR (Triglia et al 1988). This DNA was used as a probe to other mutants, and proved that in
20 independent mutations, the *Ds* had inserted into the same 6.7 kb *BglII* fragment.

The *Ds* in FT33 contains a bacterial replicon and a chloramphenicol resistance gene as a bacterial selectable marker (Rommens et al 1992). This means
25 that plant DNA carrying this transposed *Ds* can be digested with a restriction enzyme that does not cut within the *Ds* (such as *BglII*), the digestion products

can be recircularized, and then used to transform *E. coli*. Chloramphenicol resistant clones can be obtained that carry the *Ds* and adjacent plant DNA. This procedure was used to obtain a clone that carried 1.8 kb of plant DNA on the 3' side of the *Ds*, and 4.9 kb of plant DNA on the 5' side of the *Ds*.

Our present understanding of the *Cf-9* gene is depicted schematically in Figure 1. The *Cf-9* gene sequence and the deduced amino acid sequence are shown in the sequence listing.

A series of primers (F1, 2, 3, 4, 5, 6, 7, 12, 13, 10, 26, 27 and 25, indicated in Figure 1) was used to characterise a large number of independent mutations by PCR analysis in combination with primers based on the sequence of *Ds*. Therefore, these primers were used in polymerase chain reactions with primers based on the maize *Ac/Ds* transposon sequence, to characterise the locations of other mutations of *Cf-9* that were caused by transposon insertion. Eighteen independent insertions have been characterized and are located as shown. Mutants E, #55, #74 and #100 gave incomplete survival and showed a necrotic phenotype, and based on the available sequence information, they are 5' to the actual reading frame and might permit enough *Cf9* protein expression to activate an incomplete defence response.

Using the sequence obtained of the gene,

oligonucleotide primers were designed that could be used in polymerase chain reactions in combination with primers based on the sequence of the *Ds* element, to characterize both the location and the orientation of other transposon insertions in the gene. These are shown on Figure 1. Based on the results of such experiments, the map positions of 17 other *Ds* insertions have been reliably assigned (as shown in Figure 1).

10 (v) *Production of GAR plants*

On backcrossing plants that carried the *Cf-9*Ds* and *35S:SP:Avr9* gene to tomato plants that carried an *Ac* transposase gene (*sAc* that lacked the *GUS* gene) in the homozygous state, but lacked *Cf-9*, one quarter of the resulting progeny carried *sAc*, *35S:SP:Avr9* and *Cf-9*Ds* (see Figure 13) plants showed somatic excision of *Ds* from the *Cf-9*Ds* gene, somatically restoring *Cf-9* function, and giving rise to necrotic somatic sectors in which the defence response was activated.

20 Phenotypically, these plants thus showed a variegation for a defence-related necrosis, in the same manner that plants challenged with necrotizing pathogens show somatic flecks of HR that are associated with the induction of SAR.

25 Necrotic sectors were visible on cotyledons, leaves, stems, petioles, sepals, and green fruits throughout plant development. Also, the necrotic

sectors formed in both the lower and upper epidermis, in all mesophyll layers and in the cells surrounding the vascular tissue. The size of the necrotic sector and the frequency of their formation was determined by both the position of the Ds element in the *Cf-9* sequence and the orientation of the *Ds*.

The plants that variegated for necrosis were tested to assess if they were more resistant to *C. fulvum* than their unvariegated siblings that either carried *Cf-9*Ds* or carried no *Cf-9* gene. Plants from five independent *Cf-9*Ds* pedigrees were tested in which the *Ds* had independently inserted into five different locations in the *Cf-9* gene. These five independent insertions were between *Cf-9* amino acids, 7 and 8 (<M23), 28 and 29 (<M18), 47 and 48 (>M50), 56 and 57 (>M31) and 789 and 790 (>M30). The arrows (< or >) indicates the direction of transcription of the *Ds* element. F_1 plants that developed somatic necrotic sectors were more resistant to *C. fulvum* than sibling offspring that did not develop necrotic sectors. On the plants with necrotic sectors an average of 1-2 small pustules per leaf developed, 14 days after inoculation with 5×10^5 spores/ml. The plants lacking a *Cf* gene and the non variegating individuals all showed on average 38 large sporulating pustules per leaf. An example of this is shown in Figure 2.

Nine variegated *Cf-9*Ds* #20 plants, fifteen

variegated Cf-9*Ds #23 plants, eighteen variegated Cf-9*Ds #30 plants and twenty-eight variegated Cf-9*Ds #31 plants were tested, and compared to one hundred and ninety eight plants in total that did not variegate for necrosis. Plants were inoculated with *C. fulvum* (5×10^5 spores/ml) when they were four weeks old and carried 2 expanded leaves. A similar result was obtained when variegated Cf-9*Ds #50 plants and non-variegated plants were inoculated with *C. fulvum*. On 18 variegated GAR⁺ #50 plants 1-3 pustules per leaf formed, whereas on 42 non-variegated GAR⁻ #50 plants over 35 pustules per leaf developed by 14 days after inoculation.

Sensitivity to the pathogen was measured by counting the number of sporulating pustules that were visible on each genotype 14 days and 21 days after inoculation. Samples were also taken for microscopic analysis. The results of the assay after 14 days are shown in Figure 2, and typical infections on each genotype after 21 days are shown in Figure 12.

Figure 2 shows a histogram in which the sensitivity of different individual tomato plants is expressed on the y axis as the number of sporulating pustules per leaf. The Ds carried a GUS gene. M20, M23, M30 and M31 show *C. fulvum* growth on plants resulting from crosses between Cf-9*Ds and sAc, and derive from Cf-9*Ds #20, Cf-9*Ds #23, Cf-9*Ds #30 and

*Cf-9*Ds* #31, respectively. These individuals segregate from the *Cf-9*Ds* and for *sAc*. *Cf0* carries no R genes and M20, M23, M30 and M31 GUS- plants have lost by segregation both *Cf-9*Ds* and *sAc* and are thus

5 disease sensitive sibs, providing a good control for disease symptoms in sensitive individuals. If plants receive *Ds* without *sAc* they may be GUS+ without expressing the variegation for necrosis which requires both *Cf-9*Ds* and *sAc*. As can be seen, the necrotic

10 individuals (which all carry the *35S:Avr9* gene) show distinctly fewer pustules per leaf than their disease sensitive sibs.

Figure 2 shows that in these experiments, *Cf0* plants (lacking the *Cf-9* gene) exhibited about 38

15 pustules per leaf and non-variegating individuals derived from *Cf-9*Ds* #20, *Cf-9*Ds* #23 or *Cf-9*Ds* #31 also showed about 38 pustules per leaf. The non-variegated individuals that carried *Cf-9*Ds* #30 showed about 17 pustules per leaf indicating some residual

20 action of the tagged *Cf-9* allele. However, variegated individuals that carried *Cf-9*Ds* #20, *Cf-9*Ds* #23, *Cf-9*Ds* #30 or *Cf-9*Ds* #31 showed 1-3 pustules per leaf. In total seventy variegated individuals were assessed. These results demonstrate a very significant level of

25 disease control by this method.

Figure 12 shows three leaves. Leaf 1 and Leaf 2 are infected with *C. fulvum* which confers the white

fluffy appearance. Leaf 1 is Cf0 and Leaf 2 is a disease sensitive sib from Cf-9*Ds #23. Leaf 3 showing minimal sporulation is a necrotic individual (small sectors of necrosis are discernible) that carried Cf-9*Ds #23, sAc and 35S:Avr9. Leaf 3 is therefore expressing GAR.

It is important to recognize that in this example regions of variegating plants that resist the *C. fulvum* pathogen do not contain a functional Cf-9 gene. Indeed all the cells that do carry a functional Cf-9 gene (whose function was restored somatically by transposon excision) are killed as they turn on the defence response after recognition of the endogenously expressed Avr9 peptide. Thus, non-resistant cells are being induced to resistance by necrosis being manifested in adjacent cells.

EXAMPLE 2

Pathogen resistance of variegated plants employing Cf-9

In addition to demonstrating that variegated plants produced in Example 1 have enhanced resistance to *C. fulvum*, we have established that the plants are also more resistant to three unrelated fungal pathogens, *Phytophthora infestans* (the causal agent of late blight disease of tomato and potato) and *Oidium lycopersici* (a powdery mildew) and *Colletotrichum largenarium* (which causes leaf and fruit spot).

For the *P. infestans* experiments, sibling backcross progeny from the mutant *Cf-9* Ds* lines M31 and M50 that were either variegating for necrosis or not and control plants lacking a *Cf-gene* (*Cf0*) were
5 challenged by a spray application of sporangiospores (10,000 or 100 spores/ml) of the highly virulent isolate DSSI (A1 mating type). After inoculation, the plants were kept in diffuse light conditions at a constant 100% RH and 16°C and a 12h photoperiod.

10 Seven days after application of the high spore dose the leaves of the unvariegated plants and those of the *Cf0* plants were completely destroyed by the spread of *P. infestans* lesions which had abundant sporangiospores at their margins. In contrast, the
15 variegated plants were infected with *P. infestans* but the lesions were 3-5 mm in diameter and non-sporulating (Figure 3 A,B). An additional 5-6 days were required before the entire green leaf tissue of the variegated plants was destroyed and fungal sporulation commenced.

20 At the lower spore dose, by 7 days after inoculation, an average of 8-10 large sporulating lesions were present on each leaf of the unvariegated and *Cf0* plants whereas on the plants variegating for necrosis there were 1-2 small non-sporulating lesions per 10 leaves
25 (Figure 4 A,B). A minimum of 18 plants were used for each genotype/spore.

For the *Oidium lycopersici* experiments the

identical plant genotypes were used. Each leaf was inoculated by brushing with an artist paintbrush the spores from a single 14 day old sporulating pustule over an entire upper surface. The inoculated plants
5 were then kept under diffuse light conditions at 20°C during the 16 h photoperiod and at 18°C during the dark period. The RH was maintained at 70%.

By day 10 post inoculation 8-10 chlorotic lesions were evident on the leaves of the unvariegated and Cf0
10 plants and in 1-2 of these sporulation had commenced. By contrast on the variegated plants 1-2 smaller chlorotic non-sporulating lesions were present on each leaf (Figure 5). By day 14 post inoculation more than 20 sporulating lesions per leaf were present on the
15 unvariegated plants and these were accompanied by severe chlorotic symptoms on the remainder of the leaf. On the variegated plants 2-4 small sporulating lesions were present per leaf (Figure 5A). An additional 7-10 days were required before a similar level of
20 sporulation and chlorosis formed on the variegated leaves to that found on the unvariegated and Cf0 leaves at day 14 post-inoculation. (16 plants each).

EXAMPLE 3

Variegation in fruit

25 Dark green sectors formed on green tomato fruits of GAR plants, 5 weeks after flower pollination (Figure

6). These sectors were not visible once the tomato fruit had turned red, which is encouraging for potential commercial exploitation. When mature red fruit taken from GAR⁺ and GAR⁻ plants were injected with
5 100 μ l of spores of *Colletotrichum laginarium* (10⁴ spores/ml) only the GAR⁻ fruit exhibited the typical soft rot disease symptoms seven days later. Repeated inoculations of the GAR⁺ fruit failed to cause disease.

Collectively, the above results attest to a very
10 significant level of disease control that can be achieved in the plants variegating for restoration of Cf-9 gene function whilst constitutively expressing the Avr9 gene. The data also indicate that the disease control achievable by this method is potentially broad
15 spectrum because the four fungal pathogens controlled have very dissimilar modes of parasitism: *C. fulvum* is a biotroph that does not form haustoria and grows exclusively in the extracellular spaces of the leaf mesophyll layers; *O. Lycopersici* is also a biotroph but
20 colonises only the upper leaf epidermis and forms complex intracellular haustoria; *P. infestans* and *C.largenarium* are hemibiotroph that initially forms simple haustoria but later on kills host cells in both the epidermal and mesophyll layers.

25 Homozygous Cf-9*Ds, 35S:SPAvr9 lines have been established for the tomato lines M31 and M50. The F₁

backcross progeny derived from crosses to a homozygous sAc source, may be assessed for their resistance to various pathogens, including:

Potato virus X, *Pseudomonas syringae* pv. tomato,
5 Necrotrophic fungi - *Botrytis* spp, *Colletotrichum* spp,
Nematodes - *Meloidogyne incognata*, Aphids - Green Peach
Aphid, and fruit, pod, root or tuber attacking
pathogens. Also, the effect of GAR on the
establishment of mycorrhizal associations may be
10 tested.

The enhanced resistance exhibited in the plants
variegating for necrosis has been termed Genetic
Acquired Resistance (GAR). It is distinct from SAR
because it is a heritable trait and is active
15 throughout the entire plants life.

When the expression of several defence-related
genes were compared in the GAR⁻ and GAR⁺ plants,
significantly higher levels of expression of each gene
were found in the GAR⁺ plants. Examples of this are
20 shown in Figure 7 for *Cf-9*Ds* lines from M23, M31 and
M50 pedigrees using a basic tomato β -1,3 glucanase
probe and a tomato anionic peroxidase probe (pTAP 4.5).

The effectiveness of GAR in suppressing plant
disease appears to be inversely related to sector size.
25 The two independent *Cf-9*Ds* pedigrees that have the
highest frequency of small necrotic sectors (lines M31

and M50) give the best GAR. This indicates that by carefully manipulating the frequency of somatic restoration of Cf-9 function even higher levels of plant protection be developed.

5 Currently, there are two possible hypotheses to explain GAR. Either the initially activated host cells generate local and systemic signals whilst still alive, and the necrotic lesions are a by-product of the Cf-9-Avr9 mediated responses. Alternatively, the actual
10 death and necrotic reactions, the final response of the activated host cells, generates specific local and systemic signals in a manner analogous to SAR. Exactly how GAR works does not need to be known for the present invention to be operated. Provided the required
15 genetic components are present, GAR plants have enhanced pathogen resistance compared with wild-type.

EXAMPLE 4

Expression of Cf-9 in Heterologous Plants Species and Induction of Cell Necrosis

20 We have shown that following the transfer of different genomic clones containing the Cf-9 gene into tobacco and potato, these sequences render the transgenic plants responsive to Avr9 elicitor (Figure 8).

25 Also when transgenic tobacco expression Cf-9 is crossed to transgenic tobacco plants engineered to

express *Avr9* peptide constitutively, the F1 seedlings die within 2 days of seed germination (Figure 9).

When transgenic *Arabidopsis* expressing *Cf-9* is crossed to *Avr9* expressing transgenic *Arabidopsis* the F1 seedlings die 10 days after seed germination (Figure 10).

Thus we have shown that in a variety of species, genes required for activation of plant defence, mediated by the *Cf-9* protein, are present and functional.

EXAMPLE 5

Genetic Acquired Resistance Using Cf-9 in Potato

To apply GAR to potato plants a single T-DNA construct systems is used.

The system is based around a single T-DNA construct (Figure 11) containing, a *Cf-9* gene sequence under the control of its own promoter which has been inactivated by an autonomous *Ac* element that is only capable of a low level of excision (the *Ac* (Cla) element (Keller et al. 1993), and the 355:SP:*Avr9* transgene). The *Ac* element is inserted at various positions in the *Cf-9* sequence and in both orientations in order to determine the best configuration to produce a high frequency of small somatic sectors where *Cf-9* function has been restored.

Placing the *Cf-9* sequence or other R gene

sequence under the control of a cell-type specific promoter may enhance the GAR phenotype. Potential target cellular sites include the epidermis and the vascular parenchyma cells.

5 **EXAMPLE 6**

Expression of Cf-4 in transgenic plants and demonstration of increased pathogen resistance

The Cf-4 gene has been tested in transgenic plants in a number of ways: firstly by inoculation with
10 a race of *C. fulvum* containing the corresponding avirulence gene Avr4 to test if that race gives an incompatible response on the transgenic plant; secondly by injecting leaves of a transformed plant with intercellular fluid isolated from a compatible
15 interaction containing AVR4; thirdly, by delivering AVR4 in the form of recombinant potato virus X as described previously in studies of the Cf-9/AVR9 interaction (Hammond-Kosack et al., 1995).

The DNA sequence of the *C. fulvum* gene encoding
20 AVR4 has been reported and the amino acid sequence of the mature processed polypeptide (Joosten et al., 1994). We amplified by PCR the Avr4 gene from *C. fulvum* race 2,5 using primers to the published sequence and fused a sequence encoding the proposed mature
25 polypeptide to a DNA sequence encoding the N-terminal

signal peptide of the tobacco PR1a protein. This would facilitate targeting of AVR4 to the intercellular space in transgenic plants where it is expressed. This chimeric gene (SPAVr4) was inserted into a cDNA copy of potato virus X, as a *Cla*I/*Sal*I DNA fragment (SEQ ID NO. 13) as described previously (Hammond-Kosack *et al.*, 1995) to generate PVX:SPAVr4. Infectious transcripts of the recombinant virus were generated by *in vitro* transcription. All nucleic acid manipulations were performed using standard techniques well known to those skilled in the art.

Tomato

Experiments were designed to test the recombinant virus in 3 week old tomato seedlings. In *Cf*-4 containing plants inoculated cotyledons appeared desiccated and eventually abscised at 3 days post-inoculation (d.p.i.), in contrast to *Cf*0 controls which only showed signs of slight mechanical damage at the site of virus inoculation. *Cf*0 plants developed visible symptoms of virus infection at 7-10 d.p.i. comparable to symptoms observed with the wild type virus i.e. chlorotic mosaic symptoms. At 4-5 d.p.i. in plants containing *Cf*-4 necrotic lesions were observed in the younger leaves, presumably due to systemic spread of the virus as described previously in similar experiments with PVX containing *Avr*9 on *Cf*-9 containing

plants (Hammond-Kosack et al., 1995). Other features included necrotic sectors on petioles and the stem. The necrotic phenotype was seen to spread systemically and at 14 d.p.i. the majority of Cf-4 containing
5 seedlings had died. Cf0 control plants did not die but did show symptoms of chlorosis and vein-clearing.

These results confirm that Cf-4 is functional in transgenic tomato plants, resulting in a necrotic defence response in the presence of elicitor AVR4.

10 Tobacco

Using binary vector cosmids comprising Cf-4, transgenic tobacco plants have also been produced (Fillatti et al., 1987; Horsch et al., 1985) using techniques well known to those skilled in the art.

15 Transgenic tobacco containing cosmids comprising Cf-4 were inoculated with PVX:SPAVr4. In most transformants necrotic lesions were observed at the site of virus inoculation 3-4 d.p.i. similar in appearance to lesions which appear in response to virus
20 inoculation in some virus resistant varieties. In these individuals the necrosis was not strictly confined to local lesions which eventually coalesced and at 7-10 d.p.i. leaf necrosis was apparent over the entire region of virus inoculation. In several
25 transformants the reaction to PVX:SPAVr4 was more acute and the necrotic leaf sectors could be observed at 3-4

d.p.i. Neither of these phenotypes were observed in transgenic tobacco containing cosmids lacking *Cf-4* or in non-transformed control plants challenged with PVX:SPAvr4.

- 5 Functional expression of *Cf-4* in transgenic tobacco has thus also been shown, with activation of a necrotic defence response in the presence of elicitor AVR4.

Pathogen Resistance

- 10 Transgenic plants were propagated by cuttings so that *Cf-4* activity could be detected by inoculation with PVX:SPAvr4 on 12 tomato transformants. Transgenic tomato plants containing *Cf-4* exhibited leaf necrosis on inoculated leaves 3-4 d.p.i. This necrosis
15 eventually spread systemically as previously observed in *Cf-4* containing plants in the experiments described above. Transgenic plants exhibiting necrotic leaf sectors eventually died.

- Cuttings of a number of transgenic plants
20 obtained in the first round of transformation experiments were further assayed for *Cf-4* function by inoculation with *C. fulvum* race 5. In 5 transgenic plants tested, a positive correlation was observed between plants exhibiting PVX:SPAvr4 dependent necrosis
25 and resistance to the pathogen. In this experiment pathogen growth was observed on compatible control

plants (Cf0) but not on incompatible control plants (Cf2).

All documents mentioned in the text are incorporated herein by reference.

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SEQ ID NO. 1:

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AACATGCCAT GTCTGGACTC CTGCACTATC TTCCATCAAC AGGTCAATTC TCTCAACTCT	240
ATTGGTGGA GGTAGACGGT ACAAATTGAA TTATATTAAA AGACAAGCTC ACCTGAGCAT	300
CACTGTTATA CAACAACAAC AACTACGCT TCAGCCCCAA ACAATAGTGA CCCGAATCAT	360
ATATTGTCAC GAGTTTTTTT TAGAGTATGT TGCATATATT ATACTCAACT TAGGGTTTGT	420
CATTCTGATG CTTCTGTACAA ATTTATTGAA TTTTCAACTT TAAAGGTTTA TGAACCAAAT	480
ATTACGCTTA CTATGATAGC GGTCTTTTTT GATTAATCAA ACTTATTGAA TTTTCAACTT	540
TAAAGGTTTT TCCCCGTTCT ATACACAAAC TAAGAAAAAT TTAAATTATA TAGTCTTTGG	600
ATGGTGACCT ATTTGGATGG TAACATTATT GGACCAAAT ATTGATAACG CGGACATTGT	660
TAGACCAAAC TGAGAAGGAC ATGTCTGGAC TCCTGCTCCG TCTTCCATCA GCAGGTCGAT	720
TCTTGTTGGA AATTAGCTCG AGGTGGCGCA CTATGTGAGG TAAC TAGTAC TAAATTTTTT	780
TTTGCTTAAT TTGTGCTATA TATACCTCAT CTAAATTATT GAATAGTCAC ACAAAGCAAA	840
CATTCTTGA TTCTTCTCT ATCAACATAA CAAGTTTGA TCATTTTGTAG TGCAGAA	897
ATG GAT TGT GTA AAA CTT GTA TTC CTT ATG CTA TAT ACC TTT CTC TGT Met Asp Cys Val Lys Leu Val Phe Leu Met Leu Tyr Thr Phe Leu Cys -23 -20 -15 -10	945
CAA CTT GCT TTA TCC TCA TCC TTG CCT CAT TTG TGC CCC GAA GAT CAA Gln Leu Ala Leu Ser Ser Ser Leu Pro His Leu Cys Pro Glu Asp Gln -5 1 5	993
GCT CTT TCT CTT CTA CAA TTC AAG AAC ATG TTT ACC ATT AAT CCT AAT Ala Leu Ser Leu Leu Gln Phe Lys Asn Met Phe Thr Ile Asn Pro Asn 10 15 20 25	1041
GCT TCT GAT TAT TGT TAC GAC ATA AGA ACA TAC GTA GAC ATT CAG TCA Ala Ser Asp Tyr Cys Tyr Asp Ile Arg Thr Tyr Val Asp Ile Gln Ser 30 35 40	1089
TAT CCA AGA ACT CTT TCT TGG AAC AAA AGC ACA AGT TGC TGC TCA TGG Tyr Pro Arg Thr Leu Ser Trp Asn Lys Ser Thr Ser Cys Cys Ser Trp 45 50 55	1137
GAT GGC GTT CAT TGT GAC GAG ACG ACA GGA CAA GTG ATT GCG CTT GAC Asp Gly Val His Cys Asp Glu Thr Thr Gly Gln Val Ile Ala Leu Asp 60 65 70	1185
CTC CGT TGC AGC CAA CTT CAA GGC AAG TTT CAT TCC AAT AGT AGC CTC Leu Arg Cys Ser Gln Leu Gln Gly Lys Phe His Ser Asn Ser Ser Leu 75 80 85	1233
TTT CAA CTC TCC AAT CTC AAA AGG CTT GAT TTG TCT TTT AAT AAT TTC Phe Gln Leu Ser Asn Leu Lys Arg Leu Asp Leu Ser Phe Asn Asn Phe 90 95 100 105	1281
ACT GGA TCA CTC ATT TCA CCA AAA TTT GGT GAG TTT TCA AAT TTG ACG Thr Gly Ser Leu Ile Ser Pro Lys Phe Gly Glu Phe Ser Asn Leu Thr 110 115 120	1329

CAT CTC GAT TTG TCG CAT TCT AGT TTT ACA GGT CTA ATT CCT TCT GAA His Leu Asp Leu Ser His Ser Ser Phe Thr Gly Leu Ile Pro Ser Glu 125 130 135	1377
ATC TGT CAC CTT TCT AAA CTA CAC GTT CTT CGT ATA TGT GAT CAA TAT Ile Cys His Leu Ser Lys Leu His Val Leu Arg Ile Cys Asp Gln Tyr 140 145 150	1425
GGG CTT AGT CTT GTA CCT TAC AAT TTT GAA CTG CTC CTT AAG AAC TTG Gly Leu Ser Leu Val Pro Tyr Asn Phe Glu Leu Leu Lys Asn Leu 155 160 165	1473
ACC CAA TTA AGA GAG CTC AAC CTT GAA TCT GTA AAC ATC TCT TCC ACT Thr Gln Leu Arg Glu Leu Asn Leu Glu Ser Val Asn Ile Ser Ser Thr 170 175 180 185	1521
ATT CCT TCA AAT TTC TCT TCT CAT TTA ACA ACT CTA CAA CTT TCA GGC Ile Pro Ser Asn Phe Ser Ser His Leu Thr Thr Leu Gln Leu Ser Gly 190 195 200	1569
ACA GAG TTA CAT GGG ATA TTG CCC GAA AGA GTT TTT CAC CTT TCC AAC Thr Glu Leu His Gly Ile Leu Pro Glu Arg Val Phe His Leu Ser Asn 205 210 215	1617
TTA CAA TCC CTT CAT TTA TCA GTC AAT CCC CAG CTC ACG GTT AGG TTT Leu Gln Ser Leu His Leu Ser Val Asn Pro Gln Leu Thr Val Arg Phe 220 225 230	1665
CCC ACA ACC AAA TGG AAT AGC AGT GCA TCA CTC ATG ACG TTA TAC GTC Pro Thr Thr Lys Trp Asn Ser Ser Ala Ser Leu Met Thr Leu Tyr Val 235 240 245	1713
GAT AGT GTG AAT ATT GCT GAT AGG ATA CCT AAA TCA TTT AGC CAT CTA Asp Ser Val Asn Ile Ala Asp Arg Ile Pro Lys Ser Phe Ser His Leu 250 255 260 265	1761
ACT TCA CTT CAT GAG TTG TAC ATG GGT CGT TGT AAT CTG TCA GGG CCT Thr Ser Leu His Glu Leu Tyr Met Gly Arg Cys Asn Leu Ser Gly Pro 270 275 280	1809
ATT CCT AAA CCT CTA TGG AAT CTC ACC AAC ATA GTG TTT TTG CAC CTT Ile Pro Lys Pro Leu Trp Asn Leu Thr Asn Ile Val Phe Leu His Leu 285 290 295	1857
GGT GAT AAC CAT CTT GAA GGA CCA ATT TCC CAT TTC ACG ATA TTT GAA Gly Asp Asn His Leu Glu Gly Pro Ile Ser His Phe Thr Ile Phe Glu 300 305 310	1905
AAG CTC AAG AGG TTA TCA CTT GTA AAT AAC AAC TTT GAT GGC GGA CTT Lys Leu Lys Arg Leu Ser Leu Val Asn Asn Asn Phe Asp Gly Gly Leu 315 320 325	1953
GAG TTC TTA TCC TTT AAC ACC CAA CTT GAA CGG CTA GAT TTA TCA TCC Glu Phe Leu Ser Phe Asn Thr Gln Leu Glu Arg Leu Asp Leu Ser Ser 330 335 340 345	2001
AAT TCC CTA ACT GGT CCA ATT CCA TCC AAC ATA AGC GGA CTT CAA AAC Asn Ser Leu Thr Gly Pro Ile Pro Ser Asn Ile Ser Gly Leu Gln Asn 350 355 360	2049
CTA GAA TGT CTC TAC TTG TCA TCA AAC CAC TTG AAT GGG AGT ATA CCT Leu Glu Cys Leu Tyr Leu Ser Ser Asn His Leu Asn Gly Ser Ile Pro 365 370 375	2097
TCC TGG ATA TTC TCC CTT CCT TCA CTG GTT GAG TTA GAC TTG AGC AAT Ser Trp Ile Phe Ser Leu Pro Ser Leu Val Glu Leu Asp Leu Ser Asn 380 385 390	2145

AAC ACT TTC AGT GGA AAA ATT CAA GAG TTC AAG TCC AAA ACA TTA AGT Asn Thr Phe Ser Gly Lys Ile Gln Glu Phe Lys Ser Lys Thr Leu Ser 395 400 405	2193
GCC GTT ACT CTA AAA CAA AAT AAG CTG AAA GGT CGT ATT CCG AAT TCA Ala Val Thr Leu Lys Gln Asn Lys Leu Lys Gly Arg Ile Pro Asn Ser 410 415 420 425	2241
CTC CTA AAC CAG AAG AAC CTA CAA TTA CTT CTC CTT TCA CAC AAT AAT Leu Leu Asn Gln Lys Asn Leu Gln Leu Leu Leu Ser His Asn Asn 430 435 440	2289
ATC AGT GGA CAT ATT TCT TCA GCT ATC TGC AAT CTG AAA ACA TTG ATA Ile Ser Gly His Ile Ser Ser Ala Ile Cys Asn Leu Lys Thr Leu Ile 445 450 455	2337
TTG TTA GAC TTG GGA AGT AAT AAT TTG GAG GGA ACA ATC CCA CAA TGC Leu Leu Asp Leu Gly Ser Asn Asn Leu Glu Gly Thr Ile Pro Gln Cys 460 465 470	2385
GTG GTT GAG AGG AAC GAA TAC CTT TCG CAT TTG GAT TTG AGC AAA AAC Val Val Glu Arg Asn Glu Tyr Leu Ser His Leu Asp Leu Ser Lys Asn 475 480 485	2433
AGA CTT AGT GGG ACA ATC AAT ACA ACT TTT AGT GTT GGA AAC ATT TTA Arg Leu Ser Gly Thr Ile Asn Thr Thr Phe Ser Val Gly Asn Ile Leu 490 495 500 505	2481
AGG GTC ATT AGC TTG CAC GGG AAT AAG CTA ACG GGG AAA GTC CCA CGA Arg Val Ile Ser Leu His Gly Asn Lys Leu Thr Gly Lys Val Pro Arg 510 515 520	2529
TCT ATG ATC AAT TGC AAG TAT TTG ACA CTA CTT GAT CTA GGT AAC AAT Ser Met Ile Asn Cys Lys Tyr Leu Thr Leu Leu Asp Leu Gly Asn Asn 525 530 535	2577
ATG TTG AAT GAC ACA TTT CCA AAC TGG TTG GGA TAC CTA TTT CAA TTG Met Leu Asn Asp Thr Phe Pro Asn Trp Leu Gly Tyr Leu Phe Gln Leu 540 545 550	2625
AAG ATT TTA AGC TTG AGA TCA AAT AAG TTG CAT GGT CCC ATC AAA TCT Lys Ile Leu Ser Leu Arg Ser Asn Lys Leu His Gly Pro Ile Lys Ser 555 560 565	2673
TCA GGG AAT ACA AAC TTG TTT ATG GGT CTT CAA ATT CTT GAT CTA TCA Ser Gly Asn Thr Asn Leu Phe Met Gly Leu Gln Ile Leu Asp Leu Ser 570 575 580 585	2721
TCT AAT GGA TTT AGT GGG AAT TTA CCC GAA AGA ATT TTG GGG AAT TTG Ser Asn Gly Phe Ser Gly Asn Leu Pro Glu Arg Ile Leu Gly Asn Leu 590 595 600	2769
CAA ACC ATG AAG GAA ATT GAT GAG AGT ACA GGA TTC CCA GAG TAT ATT Gln Thr Met Lys Glu Ile Asp Glu Ser Thr Gly Phe Pro Glu Tyr Ile 605 610 615	2817
TCT GAT CCA TAT GAT ATT TAT TAC AAT TAT TTG ACG ACA ATT TCT ACA Ser Asp Pro Tyr Asp Ile Tyr Tyr Asn Tyr Leu Thr Thr Ile Ser Thr 620 625 630	2865
AAG GGA CAA GAT TAT GAT TCT GTT CGA ATT TTG GAT TCT AAC ATG ATT Lys Gly Gln Asp Tyr Asp Ser Val Arg Ile Leu Asp Ser Asn Met Ile 635 640 645	2913
ATC AAT CTC TCA AAG AAC AGA TTT GAA GGT CAT ATT CCA AGC ATT ATT Ile Asn Leu Ser Lys Asn Arg Phe Glu Gly His Ile Pro Ser Ile Ile 650 655 660 665	2961

75

GGA GAT CTT GTT GGA CTT CGT ACG TTG AAC TTG TCT CAC AAT GTC TTG Gly Asp Leu Val Gly Leu Arg Thr Leu Asn Leu Ser His Asn Val Leu 670 675 680	3009
GAA GGT CAT ATA CCG GCA TCA TTT CAA AAT TTA TCA GTA CTC GAA TCT Glu Gly His Ile Pro Ala Ser Phe Gln Asn Leu Ser Val Leu Glu Ser 685 690 695	3057
TTG GAT CTC TCA TCT AAT AAA ATC AGC GGA GAA ATT CCG CAG CAG CTT Leu Asp Leu Ser Ser Asn Lys Ile Ser Gly Glu Ile Pro Gln Gln Leu 700 705 710	3105
GCA TCC CTC ACA TTC CTT GAA GTC TTA AAT CTC TCT CAC AAT CAT CTT Ala Ser Leu Thr Phe Leu Glu Val Leu Asn Leu Ser His Asn His Leu 715 720 725	3153
GTT GGA TGC ATC CCC AAA GGA AAA CAA TTT GAT TCG TTC GGG AAC ACT Val Gly Cys Ile Pro Lys Gly Lys Gln Phe Asp Ser Phe Gly Asn Thr 730 735 740 745	3201
TCG TAC CAA GGG AAT GAT GGG TTA CGC GGA TTT CCA CTC TCA AAA CTT Ser Tyr Gln Gly Asn Asp Gly Leu Arg Gly Phe Pro Leu Ser Lys Leu 750 755 760	3249
TGT GGT GGT GAA GAT CAA GTG ACA ACT CCA GCT GAG CTA GAT CAA GAA Cys Gly Gly Glu Asp Gln Val Thr Thr Pro Ala Glu Leu Asp Gln Glu 765 770 775	3297
GAG GAG GAA GAA GAT TCA CCA ATG ATC AGT TGG CAG GGG GTT CTC GTG Glu Glu Glu Glu Asp Ser Pro Met Ile Ser Trp Gln Gly Val Leu Val 780 785 790	3345
GGT TAC GGT TGT GGA CTT GTT ATT GGA CTG TCC GTA ATA TAC ATA ATG Gly Tyr Gly Cys Gly Leu Val Ile Gly Leu Ser Val Ile Tyr Ile Met 795 800 805	3393
TGG TCA ACT CAA TAT CCA GCA TGG TTT TCG AGG ATG GAT TTA AAG TTG Trp Ser Thr Gln Tyr Pro Ala Trp Phe Ser Arg Met Asp Leu Lys Leu 810 815 820 825	3441
GAA CAC ATA ATT ACT ACG AAA ATG AAA AAG CAC AAG AAA AGA TAT TAGTGAGTAG Glu His Ile Ile Thr Thr Lys Met Lys Lys His Lys Lys Arg Tyr 830 835 840	3496
CTATACCTCC AGGTATTCCA CTTGATCATT ATCTTTCAGA AGATTATTTT TTGTATATCG	3556
ATGAAATTAT CGACCTCCTT CATCCTCAAA GCTCTTAACT TTCACTCTTC ATTTTGTAAA	3616
ATTTTCAGGAT TCAAAGATTT CCGAGTTCCC AGTTGCTTGG GATGCAGATA AAAGCCTTTT	3676
TATCTTTTCAT AGTTTCTTAT CCTATGAATA AAGATTTTAT TTTCAATTTGT CTATGGCAGC	3736
TAGATATGTT CCGTCACTAA AAACATTGTA TTTCTCTCAA CTCTTTCGTC ACATGATATC	3796
AAAGAACACT TGACTTCAAT TAAGTTACTG TAGTCTGCTA TTTTAATTTT TTCCATTGAA	3856
ACACAACCTGA CGTATCTTGA GAAAGAGACT ATGATCCCCC GGGCTGCAG	3905

SEQ ID NO. 2:

Met Asp Cys Val Lys Leu Val Phe Leu Met Leu Tyr Thr Phe Leu Cys
-23 -20 -15 -10

Gln Leu Ala Leu Ser Ser Ser Leu Pro His Leu Cys Pro Glu Asp Gln
-5 1 5

76

Ala Leu Ser Leu Leu Gln Phe Lys Asn Met Phe Thr Ile Asn Pro Asn
 10 15 20 25
 Ala Ser Asp Tyr Cys Tyr Asp Ile Arg Thr Tyr Val Asp Ile Gln Ser
 30 35 40
 Tyr Pro Arg Thr Leu Ser Trp Asn Lys Ser Thr Ser Cys Cys Ser Trp
 45 50 55
 Asp Gly Val His Cys Asp Glu Thr Thr Gly Gln Val Ile Ala Leu Asp
 60 65 70
 Leu Arg Cys Ser Gln Leu Gln Gly Lys Phe His Ser Asn Ser Ser Leu
 75 80 85
 Phe Gln Leu Ser Asn Leu Lys Arg Leu Asp Leu Ser Phe Asn Asn Phe
 90 95 100 105
 Thr Gly Ser Leu Ile Ser Pro Lys Phe Gly Glu Phe Ser Asn Leu Thr
 110 115 120
 His Leu Asp Leu Ser His Ser Ser Phe Thr Gly Leu Ile Pro Ser Glu
 125 130 135
 Ile Cys His Leu Ser Lys Leu His Val Leu Arg Ile Cys Asp Gln Tyr
 140 145 150
 Gly Leu Ser Leu Val Pro Tyr Asn Phe Glu Leu Leu Leu Lys Asn Leu
 155 160 165
 Thr Gln Leu Arg Glu Leu Asn Leu Glu Ser Val Asn Ile Ser Ser Thr
 170 175 180 185
 Ile Pro Ser Asn Phe Ser Ser His Leu Thr Thr Leu Gln Leu Ser Gly
 190 195 200
 Thr Glu Leu His Gly Ile Leu Pro Glu Arg Val Phe His Leu Ser Asn
 205 210 215
 Leu Gln Ser Leu His Leu Ser Val Asn Pro Gln Leu Thr Val Arg Phe
 220 225 230
 Pro Thr Thr Lys Trp Asn Ser Ser Ala Ser Leu Met Thr Leu Tyr Val
 235 240 245
 Asp Ser Val Asn Ile Ala Asp Arg Ile Pro Lys Ser Phe Ser His Leu
 250 255 260 265
 Thr Ser Leu His Glu Leu Tyr Met Gly Arg Cys Asn Leu Ser Gly Pro
 270 275 280
 Ile Pro Lys Pro Leu Trp Asn Leu Thr Asn Ile Val Phe Leu His Leu
 285 290 295
 Gly Asp Asn His Leu Glu Gly Pro Ile Ser His Phe Thr Ile Phe Glu
 300 305 310
 Lys Leu Lys Arg Leu Ser Leu Val Asn Asn Asn Phe Asp Gly Gly Leu
 315 320 325
 Glu Phe Leu Ser Phe Asn Thr Gln Leu Glu Arg Leu Asp Leu Ser Ser
 330 335 340 345
 Asn Ser Leu Thr Gly Pro Ile Pro Ser Asn Ile Ser Gly Leu Gln Asn
 350 355 360

Leu Glu Cys Leu Tyr Leu Ser Ser Asn His Leu Asn Gly Ser Ile Pro
 365 370 375
 Ser Trp Ile Phe Ser Leu Pro Ser Leu Val Glu Leu Asp Leu Ser Asn
 380 385 390
 Asn Thr Phe Ser Gly Lys Ile Gln Glu Phe Lys Ser Lys Thr Leu Ser
 395 400 405
 Ala Val Thr Leu Lys Gln Asn Lys Leu Lys Gly Arg Ile Pro Asn Ser
 410 415 420 425
 Leu Leu Asn Gln Lys Asn Leu Gln Leu Leu Leu Ser His Asn Asn
 430 435 440
 Ile Ser Gly His Ile Ser Ser Ala Ile Cys Asn Leu Lys Thr Leu Ile
 445 450 455
 Leu Leu Asp Leu Gly Ser Asn Asn Leu Glu Gly Thr Ile Pro Gln Cys
 460 465 470
 Val Val Glu Arg Asn Glu Tyr Leu Ser His Leu Asp Leu Ser Lys Asn
 475 480 485
 Arg Leu Ser Gly Thr Ile Asn Thr Thr Phe Ser Val Gly Asn Ile Leu
 490 495 500 505
 Arg Val Ile Ser Leu His Gly Asn Lys Leu Thr Gly Lys Val Pro Arg
 510 515 520
 Ser Met Ile Asn Cys Lys Tyr Leu Thr Leu Leu Asp Leu Gly Asn Asn
 525 530 535
 Met Leu Asn Asp Thr Phe Pro Asn Trp Leu Gly Tyr Leu Phe Gln Leu
 540 545 550
 Lys Ile Leu Ser Leu Arg Ser Asn Lys Leu His Gly Pro Ile Lys Ser
 555 560 565
 Ser Gly Asn Thr Asn Leu Phe Met Gly Leu Gln Ile Leu Asp Leu Ser
 570 575 580 585
 Ser Asn Gly Phe Ser Gly Asn Leu Pro Glu Arg Ile Leu Gly Asn Leu
 590 595 600
 Gln Thr Met Lys Glu Ile Asp Glu Ser Thr Gly Phe Pro Glu Tyr Ile
 605 610 615
 Ser Asp Pro Tyr Asp Ile Tyr Tyr Asn Tyr Leu Thr Thr Ile Ser Thr
 620 625 630
 Lys Gly Gln Asp Tyr Asp Ser Val Arg Ile Leu Asp Ser Asn Met Ile
 635 640 645
 Ile Asn Leu Ser Lys Asn Arg Phe Glu Gly His Ile Pro Ser Ile Ile
 650 655 660 665
 Gly Asp Leu Val Gly Leu Arg Thr Leu Asn Leu Ser His Asn Val Leu
 670 675 680
 Glu Gly His Ile Pro Ala Ser Phe Gln Asn Leu Ser Val Leu Glu Ser
 685 690 695
 Leu Asp Leu Ser Ser Asn Lys Ile Ser Gly Glu Ile Pro Gln Gln Leu
 700 705 710

78

Ala Ser Leu Thr Phe Leu Glu Val Leu Asn Leu Ser His Asn His Leu
715 720 725

Val Gly Cys Ile Pro Lys Gly Lys Gln Phe Asp Ser Phe Gly Asn Thr
730 735 740 745

Ser Tyr Gln Gly Asn Asp Gly Leu Arg Gly Phe Pro Leu Ser Lys Leu
750 755 760

Cys Gly Gly Glu Asp Gln Val Thr Thr Pro Ala Glu Leu Asp Gln Glu
765 770 775

Glu Glu Glu Glu Asp Ser Pro Met Ile Ser Trp Gln Gly Val Leu Val
780 785 790

Gly Tyr Gly Cys Gly Leu Val Ile Gly Leu Ser Val Ile Tyr Ile Met
795 800 805

Trp Ser Thr Gln Tyr Pro Ala Trp Phe Ser Arg Met Asp Leu Lys Leu
810 815 820 825

Glu His Ile Ile Thr Thr Lys Met Lys Lys His Lys Lys Arg Tyr
830 835 840

SEQ ID NO. 3:

CATTCTTGA TTTCTTCTCT ATCAACATAA CAAGTTTGA TCATTTTGTAG TGCAGAAATG 60

GATTGTGTAA AACTTGTATT CCTTATGCTA TATACCTTTC TCTGTCAACT TGCTTTATCC 120

TCATCCTTGC CTCATTTGTG CCCCGAAGAT CAAGCTCTTT CTCTTCTACA ATTCAAGAAC 180

ATGTTTACCA TTAATCCTAA TGCTTCTGAT TATTGTTACG ACATAAGAAC ATACGTAGAC 240

ATTCAGTCAT ATCCAAGAAC TCTTTCTTGG AACAAAAGCA CAAGTTGCTG CTCATGGGAT 300

GGCGTTCATT GTGACGAGAC GACAGGACAA GTGATTGCGC TTGACCTCCG TTGCAGCCAA 360

CTTCAAGGCA AGTTTCATTC CAATAGTAGC CTCTTTCAAC TCTCCAATCT CAAAAGGCTT 420

GATTTGTCTT TTAATAATTT CACTGGATCA CTCATTTTAC CAAAATTTGG TGAGTTTTC 480

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CCTTACAATT TTGAAGTGCT CCTTAAGAAC TTGACCCAAT TAAGAGAGCT CAACCTTGAA 660

TCTGTAAACA TCTCTTCCAC TATTCCTTCA AATTTCTCTT CTCATTTAAC AACTCTACAA 720

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CAATCCCTTC ATTTATCAGT CAATCCCCAG CTCACGGTTA GGTTTCCCAC AACCAAATGG 840

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CCTAAATCAT TTAGCCATCT AACTTCACCT CATGAGTTGT ACATGGGTCG TTGTAATCTG 960

TCAGGGCCTA TTCCTAAACC TCTATGGAAT CTCACCAACA TAGTGTTTTT GCACCTTGGT 1020

GATAACCATC TTGAAGGACC AATTTCCCAT TTCACGATAT TTGAAAAGCT CAAGAGGTTA 1080

TCACCTGTAA ATAACAACCT TGATGGCGGA CTTGAGTTCT TATCCTTTAA CACCCAACCT 1140

GAACGGCTAG ATTTATCATC CAATTCCTTA ACTGGTCCAA TTCCATCCAA CATAAGCGGA 1200

CTTCAAAACC TAGAATGTCT CTACTTGTCA TCAAACCACT TGAATGGGAG TATACCTTCC 1260
 TGGATATTCT CCCTTCCTTC ACTGGTTGAG TTAGACTTGA GCAATAACAC TTTCAGTGGA 1320
 AAAATTCAAG AGTTCAAGTC CAAAACATTA AGTGCCGTTA CTCTAAAACA AAATAAGCTG 1380
 AAAGGTCGTA TTCCGAATTC ACTCCTAAAC CAGAAGAACC TACAATTACT TCTCCTTTCA 1440
 CACAATAATA TCAGTGGACA TATTTCTTCA GCTATCTGCA ATCTGAAAAC ATTGATATTG 1500
 TTAGACTTGG GAAGTAATAA TTTGGAGGGA ACAATCCCAC AATGCGTGGT TGAGAGGAAC 1560
 GAATACCTTT CGCATTGTGA TTTGAGCAAA AACAGACTTA GTGGGACAAT CAATACAAC 1620
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 GTCCACGAT CTATGATCAA TTGCAAGTAT TTGACACTAC TTGATCTAGG TAACAATATG 1740
 TTGAATGACA CATTTCCAAA CTGGTTGGGA TACCTATTTT AATTGAAGAT TTTAAGCTTG 1800
 AGATCAAATA AGTTGCATGG TCCCATCAA TCTTCAGGGA ATACAAACTT GTTTATGGGT 1860
 CTTCAAATTC TTGATCTATC ATCTAATGGA TTTAGTGGGA ATTTACCCGA AAGAATTTTG 1920
 GGAATTTGC AAACCATGAA GGAAATTGAT GAGAGTACAG GATTCCCAGA GTATATTTCT 1980
 GATCCATATG ATATTTATTA CAATTATTTG ACGACAATTT CTACAAAGGG ACAAGATTAT 2040
 GATTCTGTTC GAATTTTGGA TTCTAACATG ATTATCAATC TCTCAAAGAA CAGATTTGAA 2100
 GGTCAATTC CAAGCATTAT TGGAGATCTT GTTGGACTTC GTACGTTGAA CTTGTCTCAC 2160
 AATGTCTTGG AAGGTCATAT ACCGGCATCA TTTCAAATTT TATCAGTACT CGAATCTTTG 2220
 GATCTCTCAT CTAATAAAAT CAGCGGAGAA ATTCCGCAGC AGCTTGCATC CCTCACATTC 2280
 CTTGAAGTCT TAAATCTCTC TCACAATCAT CTTGTTGGAT GCATCCCCAA AGGAAAACAA 2340
 TTTGATTCTG TCGGGAACAC TTCGTACCAA GGAATGATG GGTTACGCGG ATTTCCACTC 2400
 TCAAACTTT GTGGTGGTGA AGATCAAGTG ACAACTCCAG CTGAGCTAGA TCAAGAAGAG 2460
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 CTTGTTATTG GACTGTCCGT AATATACATA ATGTGGTCAA CTCAATATCC AGCATGGTTT 2580
 TCGAGGATGG ATTTAAAGTT GGAACACATA ATTACTACGA AAATGAAAAA GCACAAGAAA 2640
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 GGATGCAGAT AAAAGCCTTT TTATCTTTCA TAGTTTCTTA TCCTATGAAT AAAGATTTTA 2760
 TTTTCATTTG TCTATGGCAC GTAGATATGT TCCGTCATA AAAACATTGT ATTTCTCTCA 2820
 ACTCTTTCGT CACATGATAT CAAAGAACAC TTGACTTCAA TTAAGTTAAA AAAAAAAAAA 2880

SEQ ID NO. 4:

ATG GGA TTT GTT CTC TTT TCA CAA TTG CCT TCA TTT CTT CTT GTC TCT 48
 Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu Leu Val Ser
 1 5 10 15

ACA CTT CTC TTA TTC CTA GTA ATA TCC CAC TCT TGC CGT GCC TAC TGT 96
 Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg Ala Tyr Cys
 20 25 30

AAC AGT TCT TGT ACA AGA GCT TTT GAC TGT CTT GGA CAA TGT GGA AGA 144
Asn Ser Ser Cys Thr Arg Ala Phe Asp Cys Leu Gly Gln Cys Gly Arg
35 40 45

TGC GAC TTT CAT AAG CTT CAA TGT GTA CAT TGA
Cys Asp Phe His Lys Leu Gln Cys Val His 177
50 55

1	CTCGAGTTCG	GAACCTAAAA	GGTATAAAAT	ATTAATAAAA	ATTTTAAAAAT
51	GGTATATCAA	TTTTTATATT	AACCCAAACG	TCAAAATCGC	TGAAACAACA
101	GCGATTTCT	TCACCGGAAA	AAGCAAAATC	GCTACTACTG	CAGCGATTTT
151	GCAAAATGTA	ACTTTTTTTTT	AAAAAAATGC	ATATTTTCTT	ATAAGCTATA
201	TATTTGAATT	TCAAAAAAAA	TATTTGAAAA	TCAATAAAAT	TTGTTTTTCC
251	TACGATTTTC	TTTTTAAAAAT	TCTTTTTTTTG	GAAAATCCCT	ACCTAGGCAG
301	CGATTTCCAT	TTTTAATTTT	TTTTAAATAA	AAGGCAGCGA	TTTTCGAAAA
351	AAAAAATTTT	AAAAAAATTT	GAAAAAGTCG	CTGCCTAGGT	AGCGATTTGA
401	ATTTTTTTTAA	AAAATGTTAT	ATTTTGCAAA	ATCGTTGCAG	TAGCAACGAT
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501	GGTTAATATA	AAATTTTATA	TAACGTTTTG	AAATTTTTGT	TAATATTTTA
551	TAACTTTTAG	GCTCCGGACT	CAAGATTACT	CCCTCTATCT	TAGTTTATAA
601	TGCATAGTCT	GAATTTTGAA	GAGCCAAATA	GTTTAATTTT	CGCCATAAAT
651	TCAGACATGA	AATCTTTAAA	AAAGTTTAAA	TAAAATTTGT	ATATGTTGAA
701	ACTACAGAAA	AAGTATTATA	ATTCACGATA	ATTTATTAC	AAGCCATCGT
751	CGGAGTGATC	GCGAGTGAAG	TGAAAGAATT	GGAGTTTTTG	ATATCCAGAA
801	TCCATCTTGA	GAGGTTGAGA	TATCTTAATC	TATCTCCAAT	AAAAAAAAAC
851	TATTAATATC	CAATTTTCTT	GAAGGCCATT	ACCTATTCCG	ACAAATTCCA
901	CAAGATACTT	CATCATATAA	AAAAATAATC	TCCGTGAAGA	AATTCTTTTA
951	TTTGGAAAAT	CGATTTTAGA	GTCATTGCAA	TTTAATTTTA	TCAAAATATT
1001	TGAGCATGAA	AAATTTGAAA	TGGAGGTGTC	ATAAAAATAA	AATACCCTTT
1051	AAAACACGGC	TTTATTGAGT	TGACGATAGT	TCAAGTAGGG	AAAATAAATA
1101	ACTTATTAAT	TGAATATAAA	ACTTGCAAGA	AAAAAGTGAT	ATTCAAATTT
1151	AATTCTGACC	ATTATCTCTT	GATATTCTTT	GCTCTTCATT	TATTTGAATA
1201	TTCATTTTTTC	AAAAGTTCCA	CGTCATAAGA	CATCAAATAT	CAAGTAGGTC
1251	CCATAAAAAT	AAAATACCCT	TCTCAACATG	ACAAAGAAAG	ATTGAAAAAT
1301	GACTAACATT	TTCTCAAAGA	CAAAAACAAA	ACATGTGAGA	GAAGACATTA
1351	CGAATCATCA	TAATCTCTGA	GACTGAGAAT	TGTTAGATAT	GGTCCACTAC

1401 TGTAGAGATG AGAATTTTGA ACCAAATGTA TTATACACTA AGAGTGGTCA
1451 TGATCATTGT GTGATAACAA AACTATTTTG GCAACTTTGA CTCAGTCCTT
1501 GGCTAAATTA GACCTCTAAC ACAAACAAT CCAAAGTTG ACTTGAGAAT
1551 GACAACATTT TCTTCCCTGA TAGCAACCAA ATTAGCAAAT TTGGAAAAAA
1601 CGCGTGTCTT GTTGATCTTT AATTAGTATA AGTTACGTAC AATATCCTAT
1651 TGAATTGGAA ACAATAAACT CAACTATGA TGATGGTTTC TAGAAAAGTA
1701 GTCTCTTCAC TTCAGTTTTT CACTCTTTTC TACCTCTTTA CAGTTGCATT
1751 TGCTTCGACT GAGGAGGCAA CTGCCCTCTT GAAATGGAAA GCAACTTTCA
1801 AGAACCAGAA TAATTCCTTT TTGGCTTCAT GGATTCCAAG TTCTAATGCA
1851 TGCAAGGACT GGTATGGAGT TGTATGCTTT AATGGTAGGG TAAACACGTT
1901 GAATATTACA AATGCTAGTG TCATTGGTAC ACTCTATGCT TTTCCATTTT
1951 CATCCCTCCC TTCTCTTGAA AATCTTGATC TTAGCAAGAA CAATATCTAT
2001 GGTACCATT CACCTGAGAT TGGTAATCTC ACAAATCTTG TCTATCTTGA
2051 CTTGAACAAC AATCAGATTT CAGGAACAAT ACCACCACAA ATCGGTTTAC
2101 TAGCCAAGCT TCAGATCATC CGCATATTTT ACAATCAATT AAATGGATTT
2151 ATTCCTAAAG AAATAGGTTA CCTAAGGTCT CTTACTAAGC TATCTTTGGG
2201 TATCAACTTT CTTAGTGGTT CCATTCCTGC TTCAGTGGGG AATCTGAACA
2251 ACTTGTCTTT TTTGTATCTT TACAATAATC AGCTTTCTGG CTCTATTCCT
2301 GAAGAAATAA GTTACCTAAG ATCTCTTACT GAGCTAGATT TGAGTGATAA
2351 TGCTCTTAAT GGCTCTATTC CTGCTTCATT GGGGAATATG AACAACTTGT
2401 CTTTTTTGTT TCTTTATGGA AATCAGCTTT CTGGCTCTAT TCCTGAAGAA
2451 ATATGTTACC TAAGATCTCT TACTTACCTA GATTTGAGTG AGAATGCTCT
2501 TAATGGCTCT ATTCCTGCTT CATTGGGGAA TTTGAACAAC TTGTCTTTTT
2551 TGTTTCTTTA TGGAAATCAG CTTTCTGGCT CTATTCCTGA AGAAATAGGT
2601 TACCTAAGAT CTCTTAATGT CTAGGTTTG AGTGAGAATG CTCTTAATGG
2651 CTCTATTCCT GCTTCATTGG GGAATCTGAA AACTTGTCT AGGTTGAATC
2701 TTGTTAATAA TCAGCTTTCT GGCTCTATTC CTGCTTCATT GGGGAATCTG
2751 AACAAC TTGT CTATGTTGTA TCTTTACAAT AACCAGCTTT CTGGCTCTAT
2801 TCCTGCTTCA TTGGGGAATC TGAACAAC TTGTCTATGTTG TATCTTTACA
2851 ATAATCAGCT TTCTGGCTCT ATTCCTGCTT CATTGGGGAA TCTGAACAAC
2901 TTGTCTAGGT TGTATCTCTA CAATAATCAG CTTTCTGGCT CTATTCCTGA
2951 AGAAATAGGT TACTTGAGTT CTCTTACTTA TCTAGATTTG AGTAATAACT
3001 CCATTAATGG ATTTATTCCT GCTTCATTTG GCAATATGAG CAACTTGGCT
3051 TTTTGT TTC TTTATGAAAA TCAGCTTGCT AGCTCTGTTC CTGAAGAAAT

3101 AGGTTACCTA AGGTCTCTTA ATGTCCTTGA TTTGAGTGAG AATGCTCTTA
3151 ATGGCTCTAT TCCTGCTTCA TTCGGAATT TGAACAACCT GTCTAGGTTG
3201 AATCTTGTTA ATAATCAGCT TTCTGGCTCT ATTCCTGAAG AAATAGGTTA
3251 CCTAAGGTCT CTTAATGTCC TTGATTTGAG TGAGAATGCT CTTAATGGCT
3301 CTATTCCTGC TTCATTCGGG AATTTGAACA ACTTGTCTAG GTTGAATCTT
3351 GTTAATAATC AGCTTTCTGG CTCTATTCCT GAAGAAATAG GTTACCTAAG
3401 ATCTCTTAAT GACCTAGGTT TGAGTGAGAA TGCTCTTAAT GGCTCTATTC
3451 CTGCTTCATT GGGGAATCTG AACAACTTGT CTATGTTGTA TCTTTACAAT
3501 AATCAGCTTT CTGGCTCTAT TCCTGAAGAA ATAGGTTACT TGAGTTCTCT
3551 TACTTATCTA TCTTTGGGTA ATAACTCTCT TAATGGACTT ATTCCTGCTT
3601 CATTTGGCAA TATGAGAAAT CTGCAAGCTC TGATTCTCAA TGATAACAAT
3651 CTCATTGGGG AAATTCCTTC ATCTGTGTGC AATTTGACAT CACTGGAAGT
3701 GTTGTATATG CCGAGAAACA ATTTGAAGGG AAAAGTTCCG CAATGTTTGG
3751 GTAATATCAG TAACCTTCAG GTTTTGTCTG TGTCTCTAA TAGTTTCAGT
3801 GGAGAGCTCC CTTTCATCTAT TTCCAATTTA ACATCACTAC AAATACTTGA
3851 TTTTGGCAGA AACAACTCTG AGGGAGCAAT ACCACAATGT TTTGGCAATA
3901 TTAGTAGCCT CGAGGTTTTT GATATGCAGA ACAACAACT TTCTGGGACT
3951 CTTCCAACAA ATTTTAGCAT TGGATGTTCA CTGATAAGTC TCAACTTGCA
4001 TGGCAATGAA CTAGAGGATG AAATCCCTCG GTCTTTGGAC AATTGCAAAA
4051 AGCTGCAAGT TCTTGATTTA GGAGACAATC AACTCAACGA CACATTTCCC
4101 ATGTGGTTGG GAACTTTGCC AGAGCTGAGA GTTTTAAGGT TGACATCGAA
4151 TAAATTGCAT GGACCTATAA GATCATCAAG GGCTGAAATC ATGTTTCCTG
4201 ATCTTCGAAT CATAGATCTC TCTCGCAATG CATTCTCGCA AGACTTACCA
4251 ACGAGTCTAT TTGAACATTT GAAAGGGATG AGGACAGTTG ATAAAACAAT
4301 GGAGGAACCA AGTTATGAAA GCTATTACGA TGAATCGGTG GTAGTTGTGA
4351 CAAAGGGATT GGAGCTTGAA ATTGTGAGAA TTTTGTCTTT GTACACAGTT
4401 ATCGATCTTT CAAGCAACAA ATTTGAAGGA CATATTCCTT CTGTCCTGGG
4451 AGATCTCATT GCGATCCGTA TACTTAATGT ATCTCATAAT GCATTGCAAG
4501 GCTATATACC ATCATCACTT GGAAGTTTAT CTATACTGGA ATCACTAGAC
4551 CTTTCGTTTA ACCAACTTTC AGGAGAGATA CCACAACAAC TTGCTTCTCT
4601 TACGTTTCTT GAATTCTTAA ATCTCTCCCA CAATTATCTC CAAGGATGCA
4651 TCCCTCAAGG ACCTCAATTC CGTACCTTTG AGAGCAATTC ATATGAAGGT
4701 AATGATGGAT TACGTGGATA TCCAGTTTCA AAAGGTTGTG GCAAAGATCC
4751 TGTGTCAGAG AAAA ACTATA CAGTGTCTGC GCTAGAAGAT CAAGAAAGCA

4801 ATTCTGAATT TTTCAATGAT TTTTGGAAAG CAGCTCTGAT GGGCTATGGA
4851 AGTGGACTGT GTATTGGCAT ATCCATGATA TATATCTTGA TCTCGACTGG
4901 AAATCTAAGA TGGCTTGCAA GAATCATTGA AAAACTGGAA CACAAAATTA
4951 TCATGCAAAG GAGAAAGAAG CAGCGAGGTC AAAGAAATTA CAGAAGAAGA
5001 AATAATCACT TCTAGACAAG TTACCAATAC AGAAAGATTT GATTTCAGAA
5051 CTTCAGGTAT TCACGCTAAG CTCTAACACT TATCTTTTTT AGTTTATTCT
5101 AACAACTAAT ATATGGTTTT TTTTATCAAA CAAATACTTA TTAAGGCTTG
5151 ATACAAATTG CTATAATCAC TTGGAAGCTG TGATATATAA CAAAGCCTAA
5201 AAATTTATAG TTGTGTGACT CACTTTCTTA TTTTTCAGAT TTTCAGGAGC
5251 CAAGAATTAG AAGACGCTGG TGTAAAGGAT TTGCTTCTTC CTATGTTGCA
5301 GCTTATGATT GTTGGATTTG ATTTTTAGTT TTATAAGGTT TTCTTCAGTT
5351 GGGAAAATGT AATATTTTGA ATTTTGATGA TATATAATAA ATGTTGTGTA
5401 TTGAATGATG TGTATGCATT TCTCGGATCA ATAATACTCA CCTCAAAGAA
5451 TCTAAGAGAG TTAGCGCACG ATAGAAGATA GAACATACAA AGAAGAATAC
5501 ATTACAACCT TGGGCTTGGT TATCTTACAC CCCAAAGCTT GTTATTATGG
5551 AAGGAAAGGC CAAGTTTTAT TTTTAGATAT GGGGAGCCTT GGCGTGCTGG
5601 TAAGGTTGTA GTGGATAAGG TAACTTCTCC TGTTAATGAA TTGAATGATC
5651 ATAGCAGAGA TGTGTTTAAA ATTTCTGTTG TATTAGTTTG TAATATTTGG
5701 AGGTCTTAAA TTGAACAGAT GCACATCTGT TCGTGAAAGA GCATGACTAT
5751 TCTTATAAGT CAACTCTCAA GTTCTATAAA TATAAGGACT CCTAAAGTAG
5801 CATAAGAAAA AACTGCAGTA TACTAAGGCG TTGTTGGATC CTGAAGGGAA
5851 TTGCTGGTAA CCCCCTAAAC AACATACGTT ATATTGGTGG GGGGTAGAAG
5901 GTACCCAGTG AAATAATCTA GGTTCGATA GGTGCTCTG CAAACAACAA
5951 TTATTAAACA AAATCCACAC AACTAGCAC ATGAGAGTAA AAAATTTAAT
6001 GACGAGATGA AAGAACTCA CGCCAAGATG GACTTTATCA AACAACAAAT
6051 ACATTGTTTG TACCTTTTGG ACAACCATT ATCACTCAA GAAGATCAAG
6101 GATTGATGCA TTACATCGTT CTTGGAACAA AATTATGTAC ATAAAACCTA
6151 CAGGAATCAT GTTTTGTGTG TGGTAAAACT CCATAAGGAC TAGTCCAAGA
6201 TACTGAGATC AAGGATTTCT AAGTGCAGCC AATCTCTTCT CCAGTTCATC
6251 GATCCCCGAA CTGCCAGCAC GAAAGCACAA CAACAAAATG TACATGAGCG
6301 AGTTACTGAG ATCAAAGAGC ATGAAAAAAG GCACTTCATA CTAATATGAT
6351 AACTTCATAC TAATATGATA CAATTATTTA CAGGAAGAAA AGAAGAATAG
6401 GAAACCGAAC CGCAACATAC TTTATCTATT AACGAGCAGT GCACTCAAGA
6451 TAACTAGTAT TTTTGCTCGA G

SEQ ID NO. 6:

1 MMMVSRKVVS SLQFFTLFYL FTVAFASTEE ATALLKWKAT FKNQNNNSFLA
51 SWIPSSNACK DWYGVVCFNG RVNTLNITNA SVIGTLYAFP FSSLPSLENL
101 DLSKNNIYGT IPPEIGNLTN LVYLDLNNNQ ISGTIPPQIG LLAKLQIIRI
151 FHNQLNGFIP KEIGYLRSLT KLSLGINFLS GSIPASVGNL NNLSFLYLYN
201 NQLSGSIPEE ISYLRSLTEL DLSDNALNGS IPASLGNMNN LSFLFLYGNQ
251 LSGSIPEEIC YLRSLTYLDL SENALNGSIP ASLGNLNNLS FLFLYGNQLS
301 GSIPEEIGYL RSLNVLGLSE NALNGSIPAS LGNLKNLSRL NLVNNQLSGS
351 IPASLGNLNN LSMLYLYNNQ LSGSIPASLG NLNNLSMLYL YNNQLSGSIP
401 ASLGNLNNLS RLYLYNNQLS GSIPEEIGYL SSLTYLDLSN NSINGFIPAS
451 FGNMSNLAFI FLYENQLASS VPTEEIGYLR LNVLDLSENA LNGSIPASFG
501 NLNNLSRLNL VNNQLSGSIP EEIGYLRSLN VLDLSENALN GSIPASFGNL
551 NNLSRLNLVN NQLSGSIPEE IGYLRSLNDL GLSENALNGS IPASLGNLNN
601 LSMLYLYNNQ LSGSIPEEIG YLSSLTYLSL GNNSLNGLIP ASFANMRNLQ
651 ALIILNDNNLI GEIPSSVCNL TSLEVLYMPR NNLKGKVPQC LGNISNLQVL
701 SMSSNSFSGE LPSSISNLTS LQILDFGRNN LEGAIPQCFG NISSLEVFDN
751 QNNKLSGTLF TNFSIGCSLI SLNLHGNELE DEIPRSLDNC KKLQVLDLGD
801 NQLNDTFPMW LGTLPELRVL RLTSNKLHGP IRSSRAEIMF PDLRIIDLSR
851 NAFSQDLPTS LFEHLKGMRT VDKTMEEPSY ESYDDSVVV VTKGLELEIV
901 RILSLYTVID LSSNKFEGHI PSVLGDLIAI RILNVSHNAL QGYIPSSLSG
951 LSILESOLDL FNQLSGEIPQ QLASLTFLEF LNLSHNYLQG CIPQGPQFRT
1001 FESNSYEGND GLRGYPVSKG CGKDPVSEKN YTVSALEDQE SNSEFFNDFW
1051 KAALMGYGS LCGIGSMIYI LISTGNLRLW ARIIEKLEHK IIMQRRKKQR
1101 GQRNYRRRNN HF*

SEQ ID NO. 7:

1 MMMVSRKVVS SLQFFTLFYL FTVAFASTEE ATALLKWKAT FKNQNNNSFLA
51 SWIPSSNACK DWYGVVCFNG RVNTLNITNA SVIGTLYAFP FSSLPSLENL
101 DLSKNNIYGT IPPEIGNLTN LVYLDLNNNQ ISGTIPPQIG LLAKLQIIRI
151 FHNQLNGFIP KEIGYLRSLT KLSLGINFLS GSIPASVGNL NNLSFLYLYN
201 NQLSGSIPEE ISYLRSLTEL DLSDNALNGS IPASLGNMNN LSFLFLYGNQ
251 LSGSIPEEIC YLRSLTYLDL SENALNGSIP ASLGNLNNLS FLFLYGNQLS

85

301 GSIPEEIGYL RSLNVLGLSE NALNGSIPAS LGNLKNLSRL NLVNNQLSGS
 351 IPASLGNLNN LSMLYLYNNQ LSGSIPASLG NLNNLSMLYL YNNQLSGSIP
 401 ASLGNLNNLS RLYLYNNQLS GSIPEEIGYL SSLTYLDLSN NSINGFIPAS
 451 FGNMSNLAFL FLYENQLASS VP EEIGYLRS LNVLDLSENA LNGSIPASFG
 501 NLNNLSRLNL VNNQLSGSIP EEIGYLRS LN VLDLSENALN GSIPASFGNL
 551 NNLSRLNLVN NQLSGSIP EEIGYLRS LN DL GLSENALNGS IPASLGNLNN
 601 LSMLYLYNNQ LSGSIP EEIG YLSSLTYLSL GNNSLNLGLIP ASFANMRNLQ
 651 ALILNDNNLI GEIPSSVCNL TSLEVLYMPR NNLKGKVPQC LGNISNLQVL
 701 SMSSNSFSGE LPSSISNLTS LQILD FGRNN LEGAIPQCFG NISSLEVFD
 751 QNNKLSGTLT TNFSIGCSLI SLNLHGNELE DEIPRSLDNC KKLQVLDLGD
 801 NQLNDTFPMW LGTLP ELRVL RLTSNKLHGP IRSSRAEIMF PDLRIIDL
 851 NAFSODLPTS LFEHLKGMRT VDKTMEEPSY ESYDDSVVV VTKGLELEIV
 901 RILSLTYTVID LSSNKFEGHI PSVLGDLIAI RILNVSHNAL QGYIPSSLGS
 951 LSILES LDLS FNQLSGEIPQ QLASLTFLEF LNL SHNYLQG CIPQGPQFRT
 1001 FESNSYEGND GLRGYPVSKG CGKDPVSEKN YTVSALEDQE SNSEFFNDFW
 1051 KAALMGYGS LCIGISIIYI LISTGNLRLW ARIIEELEHK IIMORRKKOR
 1101 GQRNYRRRNN RF*

SEQ ID NO. 8:

1 GGTTCCTAGA AAAGTAGTCT CTTCACTTCA GTTTTTCCTACT CTTTCTTACC
 51 TCTTTACAGT TGCATTTGCT TCGACTGAGG AGGCAACTGC CCTCTTGAAA
 101 TGGAAAGCAA CTTTCAAGAA CCAGAATAAT TCCTTTTGG CTTTCATGGAT
 151 TCCAAGTTCT AATGCATGCA AGGACTGGTA TGGAGTTGTA TGCTTTAATG
 201 GTAGGGTAAA CACGTTGAAT ATTACAAATG CTAGTGTCAT TGGTACACTC
 251 TATGCTTTTC CATTTTCATC CCTCCCTTCT CTTGAAAATC TTGATCTTAG
 301 CAAGAACAAT ATCTATGGTA CCATTCCACC TGAGATTGGT AATCTCACAA
 351 ATCTTGCTTA TCTTGACTTG AACAAACAATC AGATTTTCAGG AACAAATACCA
 401 CCACAAATCG GTTTACTAGC CAAGCTTCAG ATCATCCGCA TATTTACAA
 451 TCAATTAAAT GGATTTATTC CTAAAGAAAT AGGTACCTA AGGTCTCTTA
 501 CTAAGCTATC TTTGGGTATC AACTTTCTTA GTGGTTCCAT TCCTGCTTCA
 551 GTGGGGAATC TGAACAACTT GTCTTTTGTG TATCTTTACA ATAATCAGCT
 601 TTCTGGCTCT ATTCTGAAG AAATAAGTTA CCTAAGATCT CTTACTGAGC
 651 TAGATTTGAG TGATAATGCT CTTAATGGCT CTATTCCTGC TTCATTGGGG
 701 AATATGAACA ACTTGCTTTT TTTGTTTCTT TATGGAAATC AGCTTTCTGG

751 CTCTATTCCT GAAGAAATAT GTTACCTAAG ATCTCTTACT TACCTAGATT
801 TGAGTGAGAA TGCTCTTAAT GGCTCTATTC CTGCTTCATT GGGGAATTTG
851 AACAACTTGT CTTTTTTTGT TCTTTATGGA AATCAGCTTT CTGGCTCTAT
901 TCCTGAAGAA ATAGGTTACC TAAGATCTCT TAATGTCCTA GGTGAGTG
951 AGAATGCTCT TAATGGCTCT ATTCCTGCTT CATTGGGGAA TCTGAAAAC
1001 TTGTCTAGGT TGAATCTTGT TAATAATCAG CTTTCTGGCT CTATTCCTGC
1051 TTCATTGGGG AATCTGAACA ACTTGTCTAT GTTGTATCTT TACAATAACC
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1151 ATGTTGTATC TTTACAATAA TCAGCTTTCT GGCTCTATTC CTGCTTCATT
1201 GGGGAATCTG AACAACTTGT CTAGGTTGTA TCTCTACAAT AATCAGCTTT
1251 CTGGCTCTAT TCCTGAAGAA ATAGGTTACT TGAGTTCTCT TACTTATCTA
1301 GATTGAGTA ATAACCTCCAT TAATGGATTT ATTCCTGCTT CATTGCGCA
1351 TATGAGCAAC TTGGCTTTTT TGTTCCTTTA TGAAAATCAG CTTGCTAGCT
1401 CTGTTCTCTGA AGAAATAGGT TACCTAAGGT CTCTTAATGT CCTTGATTTG
1451 AGTGAGAATG CTCTTAATGG CTCTATTCCT GCTTCATTCG GGAATTTGAA
1501 CAACTTGTCT AGGTTGAATC TTGTTAATAA TCAGCTTTCT GGCTCTATTC
1551 CTGAAGAAAT AGGTTACCTA AGGTCTCTTA ATGTCCTTGA TTTGAGTGAG
1601 AATGCTCTTA ATGGCTCTAT TCCTGCTTCA TTCGGGAATT TGAACAACCT
1651 GTCTAGGTTG AATCTTGTTA ATAATCAGCT TTCTGGCTCT ATTCCTGAAG
1701 AAATAGGTTA CCTAAGATCT CTTAATGACC TAGGTTTGAG TGAGAATGCT
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1801 GTTGTATCTT TACAATAATC AGCTTTCTGG CTCTATTCCT GAAGAAATAG
1851 GTTACTTGAG TTCTCTTACT TATCTATCTT TGGGTAATAA CTCTCTTAAT
1901 GGACTTATTC CTGCTTCATT TGGCAATATG AGAAATCTGC AAGCTCTGAT
1951 TCTCAATGAT AACAACTCTA TTGGGGAAAT TCCTTCATCT GTGTGCAATT
2001 TGACATCACT GGAAGTGTTG TATATGCCGA GAAACAATTT GAAGGGAAAA
2051 GTTCCGCAAT GTTTGGGTAA TATCAGTAAC CTTCAGGTTT TGTCGATGTC
2101 ATCTAATAGT TTCAGTGGAG AGCTCCCTTC ATCTATTTCC AATTTAACAT
2151 CACTACAAAT ACTTGATTTT GGCAGAAACA ATCTGGAGGG AGCAATACCA
2201 CAATGTTTTG GCAATATTAG TAGCCTCGAG GTTTTTGATA TGCAGAACAA
2251 CAACTTTTCT GGGACTCTTC CAACAAATTT TAGCATTGGA TGTTCACTGA
2301 TAAGTCTCAA CTTGCATGGC AATGAACTAG AGGATGAAAT CCCTCGGTCT
2351 TTGGACAATT GCAAAAAGCT GCAAGTTCTT GATTTAGGAG ACAATCAACT
2401 CAACGACACA TTTCCCATGT GGTTGGGAAC TTTGCCAGAG CTGAGAGTTT

2451 TAAGGTTGAC ATCGAATAAA TTGCATGGAC CTATAAGATC ATCAAGGGCT
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 2551 CTCGCAAGAC TTACCAACGA GTCTATTTGA ACATTTGAAA GGGATGAGGA
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 3001 CAATTCATAT GAAGGTAATG ATGGATTACG TGGATATCCA GTTTCAAAAG
 3051 GTTGTGGCAA AGATCCTGTG TCAGAGAAAA ACTATACAGT GTCTGCGCTA
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 3151 TCTGATGGGC TATGGAAGTG GACTGTGTAT TGGCATATCC ATAATATATA
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 3251 CTGGAACACA AAATTATCAT GCAAAGGAGA AAGAAGCAGC GAGGTCAAAG
 3301 AAATTACAGA AGAAGAAATA ATCGCTTCTA GACAAGTTAC CAATACCGAA
 3351 AGATTTGATT TCAGAACTTC AGACTTTCAG GAGCCAAGAA TAAGAAGACG
 3401 CTGGTGTAAG GGATTTGCTT CTTCTGTGTG TGCAGCTTAT GATGTTGGAT
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 3551 AAAAAAAAAA AAAAAAAAAA AAA

SEQ ID NO. 9:

1 tatatatctt aataatgtaa attgatgaca aagtgattaa atagatgac
 51 gtgagagatg aaatcaggta gagttttgtg ttgttgtttc aggaattata
 101 cgagtcaagg tacttgagg ggatggagtt gagaaaatgg ggcgaacgca
 151 acacaaaaag cagagagttt ctagacgcaa ttccacggcc gcttcttgaa
 201 ctcgttgata gatgtttgat agttaaccgg aggcgacgaa tcagcgcaga
 251 ggatgctctc aagcacgagt tcttctatcc agtacatgaa acccttagaa
 301 accaaatgct ccttaaacag cagcaaatgc aatcgcagcc tacagttgtt
 351 gctgacgcac taagcgaac tttaaactaa ttatacaatt cttaaaaact
 401 aaaagagtaa tttagcaaac tagagagtta attttcactt tagcaaacta
 451 gagagttaat ttaatttagc gaactaatta tattttcact ttagtatata
 501 attcttagtg ttaatttagt attttcactt atattatttg aattaaaaatc
 551 ctcataatcg atatacttat tctcctaate catgtgcatg tatgtattgg
 601 gaaacaagac tttgatatta aacaatcata agtacattct tacgataaaa
 651 tgccttgtag aaggacaact gacaccaca aaatatgtgt gtttcaaaat
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 751 gaaatattat ttctgtcttg tacaaagact aagacttatc ataattaagt
 801 gacaaccaca aaaattcaat ctctaaaaat atctttgtat gtagtgtaaa

851	aaagcttttcg	aggaaaagtaa	gacgaagttt	ctcctctctt	tctcacacta
901	tgtcttgctg	atttacttct	cttaaaaaatc	ttcgtctctt	ctctgagttc
951	gctctatcat	ctcccATGGC	GGCTTCTTCT	TCTTCTGGCA	GACGGAGATA
1001	CGACGTTTTT	CCAAGCTTCA	GTGGGGTTGA	TGTTTCGCAAG	ACGTTCCCTCA
1051	GCCATCTTCT	CAAGGCTCTC	GACGGCAAAT	CAATCAATAC	ATTTCATCGAT
1101	CATGGAATCG	AGAGAAGCCG	CACAATCGCC	CCTGAGCTTA	TATCGGCGAT
1151	TAGAGAAGCT	AGGATCTCAA	TCGTCTCTT	CTCTAAGAAC	TATGCTTCTT
1201	CAACGTGGTG	CTTAAATGAA	TTGGTTGAGA	TCCACAAGTG	CTTTAATGAT
1251	TTAGGTCAAA	TGGTGATTCC	AGTTTTCTAC	GACGTTGATC	CTTCGGAAAGT
1301	TAGAAAACAG	ACCGGCGAAT	TTGGAAAGGT	CTTTGAAAAG	ACATGCGAGG
1351	TCAGCAAGGA	CAAACAACCA	GGGGATCAGA	AACAAAGATG	GGTGCAAGCT
1401	CTCACAGATA	TAGCAAATAT	AGCCGGAGAG	GATCTTCTGA	ACGGgtacgt
1451	tgttatgatt	ccaatatatc	tgcttgctgt	ttcaattgtc	tcagaactat
1501	atthttgcat	agacttcggt	tcttctttta	gggtgcttc	ttaattgaca
1551	aaattgactt	ttgttattag	GCCTAATGAA	GCGCATATGG	TTGAAAAGAT
1601	ATCCAATGAT	GTTTCGAATA	AACTTATCAC	TCGGTCAAAG	TGTTTTGATG
1651	ACTTCGTCCG	AATTGAAGCT	CATATTGAGG	CAATAAAATC	AGTATTGTGC
1701	TTGGAATCCA	AGGAAGCTAG	AATGGTCGGG	ATTTGGGGAC	AGTCAGGGAT
1751	TGGTAAGAGT	ACCATCGGAA	GAGCTCTTTT	CAGTCAACTC	TCTAGCCAGT
1801	TCCACCATCG	CGCTTTCCTA	ACTTATAAAA	GCACCAAGTG	TAGTGACGTC
1851	TCTGGCATGA	AGTTGAGTTG	GCAAAAAGAG	CTTCTCTCGG	AAATCTTAGG
1901	TCAAAAGGAC	ATAAAGATAG	AGCATTTTGG	TGTGGTGGAG	CAAAGGTTAA
1951	ATCACAAGAA	AGTTCCTTATC	CTTCTTGATG	ATGTGGATAA	TCTAGAGTTT
2001	CTTAAGACCT	TGGTGGGAAA	AGCTGAATGG	TTTGGATCTG	GAAGCAGAAT
2051	AATTGTGATC	ACTCAAGATA	GGCAACTTCT	CAAGGCTCAT	GAGATTGACC
2101	TTGTATATGA	GGTGAAGCTG	CCATCTCAAG	GTCTTGCTCT	TAAGATGATA
2151	TCCCAATATG	CTTTTGGGAA	AGACTCTCCA	CCTGATGATT	TTAAGGAACT
2201	AGCATTTGAA	GTTGCCGAGC	TTGTCGGTAG	TCTTCCTTTG	GGTCTCAGTG
2251	TCTTGGGTTT	ATCTTTAAAA	GGAAGGGACA	AAGATGAGTG	GGTGAAGATG
2301	ATGCCTAGGC	TTCGAAATGA	TTCCAGATGAT	AAAATTGAGG	AAACACTAAG
2351	AGTCGGCTAC	GATAGGTTAA	ATAAAAAAAA	TAGAGAGTTA	TTTAAGTGCA
2401	TTGCATGTTT	TTTCAATGGT	TTTAAAGTCA	GTAACGTCAA	AGAATTACTT
2451	GAAGATGATG	TTGGGCTTAC	AATGTTGGCT	GAGAAGTCCC	TCATACGTAT
2501	TACACCGGGT	GGATATATAG	AGATGCACAA	TTTGCTAGAG	AAATTGGGTA
2551	GAGAAATTGA	TCGTGCAAAG	TCCAAGGGTA	ATCCTGGAAA	ACGTCAATTT
2601	CTGACGAATT	TTGAGGATAT	TCGAGAAGTA	TTGACCGAGA	AAACTgtaag
2651	tttttcgcat	ctccttaaac	gttgtaatgc	atgactttat	atcaatataa
2701	tcgtaaatgg	gggattgata	aacttaagca	attggtgccc	catgcgtaat
2751	taaaacgtag	ctttgatgtg	tcagaaaaat	aaaaagggtt	gcgattgtta
2801	agattatatt	agttttcttc	ggattttttt	tcagGGGACC	GAAACTCTTC
2851	TTGGAATACG	TTTGCCACAC	CCGGGATATC	TTACGACAAG	GTCGTTCTTA
2901	ATAGATGAAA	AATCATTCAA	AGGCATGCGT	AATCTCCAAT	ATCTAGAAAT
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3001	GTAACCTCAA	AAGGCTATGG	TGGGATAATT	GTCCATTGAA	GCGTTTGCCT
3051	TCTAATTTTA	AGGCTGAGTA	TCTGGTTGAA	CTCAGAATGG	TGAATAGTAA
3101	GCTTGAGAAG	CTGTGGGATG	GAACCTCAGG	actaatTTTT	ttagtgatca
3151	atttctaaac	ataaaaaacta	aaaataaaaa	tgtttaaaat	gttcattaac
3201	gtgtgtgctc	tcttttcccc	tattttgttt	tcagCCCCTT	GGAAGTCTCA
3251	AGAAGATGGA	TTTGTATAAT	TCCTACAAAT	TGAAAGAAAT	TCCAGATCTT
3301	TCTTTAGCCA	TAAACCTCGA	GGAATTAAAT	CTTGAAGAAT	GCGAATCTTT
3351	GGAGACACTT	CCTTCCTCGA	TTCAGAATGC	CATTAAACTG	AGGGAGTTAA
3401	ATTGTTGGGG	GGGGCTATTA	ATAGATTTAA	AATCATTAGA	AGGCATGTGT
3451	AATCTCGAAT	ATCTATCAGT	TCCTAGTTGG	TCAAGTAGGG	AATGCACTCA
3501	GGGCATCGTT	TATTTCCCTC	GTAAACTCAA	AAGTGTATTG	TGGACTAATT
3551	GTCCATTGAA	GCGTTTGCCT	TCTAATTTTA	AGGCTGAGTA	TCTGGTTGAA
3601	CTCATAATGG	AGTACAGTGA	GCTTGAGAAG	CTGTGGGATG	GTAAGTCAAG
3651	actaattcta	ttagtataaa	taaatatgtt	agaaaaacta	aaaataaaaa
3701	tgtttaaaat	gttcattaac	gtgtgtgctc	tcttttcccc	tattttgtta
3751	tcagTCACTT	GGAAGTCTCA	AGGAGATGAA	TTTGAGGTAT	TCCAACAATT
3801	TAAAAGAAAT	TCCAGATCTT	TCTTTAGCCA	TAAACCTCGA	GGAATTAGAT
3851	CTTTTGGAT	GCGTATCTTT	GGTGACACTT	CCTTCCTCGA	TTCAGAATGC
3901	CACTAAACTG	ATCTATTTAG	ATATGAGTGA	ATGCGAAAAT	CTAGAGAGTT
3951	TTCCAACCGT	TTTCAACTTG	AAATCTCTCG	AGTACCTCGA	TCTCACTGGA
4001	TGCCCCGAAT	TGAGAAATTT	CCCAGCAATC	AAAATGGGAT	GTGCCTGGAC
4051	TAGATTATCT	CGAACAAGAT	TGTTTCCGGA	AGGGAGAAAT	GAGATCGTGG
4101	TAGAAGATTG	TTTCTGGAAC	AAGAATCTCC	CTGCTGGACT	AGATTATCTC
4151	GACTGCCTTA	TGAGATGTAT	GCCTTGTGAA	TTTCGCTCAG	AACAACCTCAC

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4201 TTTTCTCAAT GTGAGCGGCT GCAAGCTTGA GAAGCTATGG GAAGGCATCC
4251 AGgtacattg ttaatgctat gctgattttt gtttaccttc tgttatataa
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4501 TCTGAAAACC TGAAAGAACT TCCAGATCTT TCAAAGGCCA CCAATCTGAA
4551 GCTTTTATGT CTCAGCGGGT GCAAAAGTTT GGTGACACTT CCTTCTACAA
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5901 ataaatttat aatgataatg acaaaacgat ttcatagggt ttgacttttg
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SEQ ID NO. 10:

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1  MAASSSSGRR RYDVFPFSFG VDVRKTFLSH LLKALDGKSI NTFIDHGIER
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101  IPVFYDWDPS EVRKQTGEFG KVFEKTCEVS KDKQPGDQKQ RWVQALTDIA
151  NIAGEDLLNG PNEAHMVEKI SNDVSNKLIT RSKCFDDFVG IEAHIEAIKS
201  VLCLESKEAR MVGIWQSGI GKSTIGRALF SQLSSQFHHR AFLTYKSTSG
251  SDVSGMKLSW QKELLSEILG QKDIKIEHFG VVEQRLNHKK VLILLDDVDN
301  LEFLKTLVGK AEWFGSGSRI IVITQDRQLL KAHEIDLVEY VKLPSQGLAL
351  KMISQYAFGK DSPPDDFKEL AFEVAELVGS LPLGLSVLGS SLKGRDKDEW
401  VKMMPRLRND SDDKIEETLR VGYDRNLNKN RELFKCIACF FNGFKVSNVK
451  ELLEDDVGLT MLAEKSLIRI TPGGYIEMHN LLEKLGREID RAKSKGNPGK
501  RQFLTNFEDI REVLTEKTGT ETLLGIRLPH PGYLTTRSFL IDEKSFKGMR
551  NLQYLEIGYW SDGVLPQSLV YFPRKLKRLW WDNCPKRLP SNFKAEYLVE
601  LRMVNSKLEK LWDGTQPLGS LKKMDLYNSY KLKEIPDLSL AINLEELNLE
651  ECESLETLPs SIGNAIKLE LNCWGGLLID LKSLEGMCNL EYLSVPSWSS

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90

701 RECTQGIVYF PRKLKSVLWT NCPLKRLPSN FKAEYLEVELI MEYSELEKLW
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 801 QNATKLIYLD MSECENLESF PTVFNLKSLE YDLTGCPNL RNFPKIMGC
 851 AWTRLRTRL FPEGRNEIVV EDCFVNKNLP AGLDYLDCLM RCMPCEFRSE
 901 QLTFLNVSGC KLEKLWEGIQ SLGSLEEMDL SESENKELP DLSKATNLKL
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 1001 SGCSSLRTFP LISTNIVCLY LENTAIEEIP DLSKATKLES LIINCKSLV
 1051 TLPSTIGNLQ NLRRLYMNR TGLELLPTDV NLSSLETLDL SGCSSLRTFP
 1101 LISTRIECLY LENTAIEEVP CCIEDFTRLT VLRMYCCQRL KNISPNIFRL
 1151 TSLTLADFTD CRGVIKALSD ATVVATMEDH VSCVPLSENI EYTCERFWD
 1201 CSDYYSDDFE VNRNPIRLST MTVNDVEFKF CCSITIKECG VRLLYVYQET
 1251 EHNQOTTRSK KMRVSLLP

SEQ ID No.11:

1 GACCAAACCTG GACTCCTGCT CCGTCTTCCA TCAGCAGGTC AATTCTCGTG
 51 GAAAATTAGC TCGAGGTGGC GCACTATGTG AGGTAGCTAG TACTAAATGT
 101 TTATTTGCGT AATTTGTGCT ATATATACCT CATCTAAATT ATTGAATAGA
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 201 ATGGGTTGTG TAAAACTTGT GTTTTTCATG CTATATGTCT TTCTCTTCA
 251 ACTTGTTTCC TCGTCATCCT TACCTCATTT GTGCCCCGAA GATCAAGCTC
 301 TTGCTCTTCT AGAATTCAAG AACATGTTTA CCGTTAATCC TAATGCTTCT
 351 GATTATTGTT ACGACAGAAG AACTCTTCT TGAACAAAA GCACAAGTTG
 401 CTGCTCATGG GATGGCGTTC ATTGTGACGA AACGACAGGA CAAGTGATTG
 451 AGCTTGACCT CCGTTGCATC CAACTTCAAG GCAAGTTTCA TTCCAATAGT
 501 AGCCTCTTTC AACTCTCCAA TCTCAAAGG CTTGATTTGT CTTATAATGA
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1951 CTGATCCATA TGATATTTAT TACAATTATT TGACGACAAT TTCTACAAAG
2001 GGACAAGATT ATGATTCTGT TCGAATTTTG GATTCTAACA TGATTATCAA
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2151 ATACCGGCAT CATTTCAAAA TTTATCAGTA CTCGAATCAT TGGATCTCTC
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2301 AAAGGAAAAC AATTTGATTC GTTCGGAAC ACTTCGTACC AAGGGAATGA
2351 TGGGTACGC GGATTTCCAC TCTCAAACT TTGTGGTGGT GAAGATCAAG
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2601 AAGCACAAGA AAAGATATTA GTGAGTAGCT ATACCTCCAG GTATTCCACT
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2701 ACCTCCTTCA TCCTCAAAGC TCTTAACTTT CACTCTTCAT TTTTGAAAAT
2751 TTCAGGATTC AAAGATTTCC GAGTTCCCAG TTGCTTGGGA TGCAGATAAA
2801 AGCCTTTTTA TCTTTCATAG TTTCTTATCC TATGAATAAA GATTTTATTT
2851 TCATTTGTCT ATGGCACGTA GATATGTTCC GTCATAAAA ACATTGTATT
2901 TCTCTCAACT CTTTCGTCAC ATGATATCAA AGAACACTTG ACTTCAATTA
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3001 TATCTTGAGA AAGAGACTAT GATCTCAGAA ATGGGAATCT CCCAATCCAA

SEQ ID No. 12:

1 MGCVKLVFFM LYVFLFQLVS SSSLPHLCPE DQALALLEFK NMFTVNPNAS
51 DYC YDRRTL S WNKSTSCCSW DGVHCDETTQ QVIELDLRCI QLQGFHSNS
101 SLFQLSNLKR LDLSYNDFTG SPISPKFGEF SDLTHLDLSH SSFRGVIPSE
151 ISHLSKLYVL RISLNELTFG PHNFELLLKN LTQLKVLDE SINISSTIPL
201 NFSSHLTNLW LPYTELRGIL PERVFHLSDL EFLDLSSNPQ LTVRFPTTKW
251 NSSASLMKLY LYNVNIDDRI PESFSLTSL HKLYMSRSNL SGPIPKPLWN
301 LTNIVFLDLN NNHLEGPIPS NVSGLRNLQI LWLSSNNLNG SIPSWIFSLP
351 SLIGLDLSNN TFSGKIQEFK SKTLSTVTLK QNKLGPIPN SLLNQKNLQF
401 LLLSHNNISG HISSAICNLK TLILLDLGSN NLEGTIPQCV VERNEYLSHL
451 DLSNNRLSGT INTTFSVGNL LRVISLHGNK LTGKVPRSMI NCKYLTLLDL
501 GNNMLNDTFP NWLG YLFQLK ILSLRSNKLH GPIKSSGNTN LFMGLQILD L
551 SSNGFSGNLP ERILGNLQTM KEIDESTGFP EYISDPYDIY YNYLTITSTK
601 GQDYDSVRIL DSNMIINLSK NREFEGHIPSI IGDVLGLRTL NLSHNVLEGH
651 IPASFQNL SV LESLDLSSNK ISGEIPQQLA SLTFLEVLNL SHNHLVGCIP
701 KGKQFDSFGN TSYQGNDGLR GFPLSKLCGG EDQVTTPAEL DQEEEEEDSP
751 MISWQGVLVG YGCGLVIGLS VIYIMWSTQY PAWFSRMDLK LEHIITTKMK
801 KHKKRY

1 ATCGATGGGATTGTTCTCTTTTCACAATTGCCTTCATTTCTTCTTGTCTCTACACTTCT 60
-----+-----+-----+-----+-----+-----+
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M G F V L F S Q L P S F L L V S T L L
61 CTTATTTCCTAGTAATATCCCACTCTTGCCGTGCCAAAGCCCCCAAACTCAACCATACAA 120
-----+-----+-----+-----+-----+-----+
GAATAAGGATCATTATAGGGTGAGAACGGCACGGTTTCGGGGGTTTGTGAGTTGGTATGTT
L F L V I S H S C R A K A P K T Q P Y N
121 CCCATGCAAGCCCCAAGAAGTCATCGACACCAAGTGTATGGGTCCCAAGGATTGTCTCTA 180
-----+-----+-----+-----+-----+-----+
GGGTACGTTTCGGGGTCTTCAGTAGCTGTGGTTCACATACCCAGGGTTCCTAACAGAGAT
P C K P Q E V I D T K C M G P K D C L Y
181 CCCGAACCCCGACAGTTGTACAACCTACATACAGTGTGTACCGCTCGACGAAGTTGGCAA 240
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P N P D S C T T Y I Q C V P L D E V G N
241 TGCGAAGCCTGTGGTTAAGCCATGTCCAAAAGGACTGCAGTGGAAACGATAACGTTGGCAA 300
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ACGCTTCGGACACCAATTCGGTACAGGTTTTCTGACGTCACCTTGCTATTGCAACCGTT
A K P V V K P C P K G L Q W N D N V G K
301 GAAGTGGTGC GACTATCCAAACCTGAGTACGTGTCCGGTAAAGACGCCGCAACCGAAGCC 360
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CTTCACCACGCTGATAGGTTTGGACTCATGCACAGGCCATTTCTGCGGCGTTGGCTTCGG
K W C D Y P N L S T C P V K T P Q P K P
361 GAAGAAGGGAGGTGTCCGAGGGAAGAAGGCGTCGGTTGGACATCCTGGCTA^{MT}TGAGTCGG 420
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421 ACAAGAAAGGGGATGGCTGTAACAGTTCTGGTACCAGAGCTATCGTGCTAGGGGATCCGT 480
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481 ----
GCTG

CLAIMS:

1. A method of providing increased pathogen resistance in a plant, or a part or propagule of a plant, by induction of variegation in which a gene is expressed or suppressed in cells resulting in the activation of a plant defence response, which comprises:
- (i) inactivating a nucleotide sequence which contributes to a plant defence response or inactivating one or more nucleotide sequences forming a part of a combination of nucleotide sequences which contributes to a plant defence response;
- (ii) introducing said nucleotide sequence or sequences into the genome of a plant; and
- (iii) restoring said nucleotide sequence or sequences to a functional form in cells of the plant or a descendant thereof, or a part or propagule of the plant or descendant, to result in increased pathogen resistance.
2. A method of providing increased pathogen resistance in a plant, or a part or propagule thereof, by induction of variegation in which a gene is expressed or suppressed resulting in necrosis, which comprises:
- (i) inactivating a nucleotide sequence which contributes to necrosis or inactivating one or more nucleotide sequences forming part of a combination of

nucleotide sequences which contributes to necrosis;
(ii) introducing said nucleotide sequence or
sequences into the genome of a plant; and
(iii) restoring said inactivated nucleotide sequence or
5 sequences to a functional form in cells of the plant or
a descendant thereof, or a part or propagule of the
plant or descendant, to result in necrosis.

3. A method according to claim 1 or claim 2 wherein
said nucleotide sequence encodes or sequences encode a
10 substance or a combination of substances which result
in increased pathogen resistance.

4. A method according to any one of the preceding
claims wherein said nucleotide sequence or sequences
comprises a gene and activation of the plant defence
15 response and/or necrosis due to the expression of said
nucleotide sequence or sequences is not dependent on
the expression of any other gene comprised in said
nucleotide sequence or sequences.

5. A method according to any one of claims 1 to 3
20 wherein said nucleotide sequence or combination of
nucleotide sequences comprises one or more genes and
wherein activation of the plant defence response and/or
necrosis due to the expression of said nucleotide
sequence or sequences is conditional on the expression
25 of one or more interacting genes.

6. A method according to claim 5 wherein said nucleotide sequences encodes or nucleotide sequences encode one or more substances which are or together are capable of inducing the plant defence response and/or
5 necrosis, and at least one of said nucleotide sequences is inactivated in step (i).

7. A method according to claim 6 wherein said nucleotide sequence comprises a plant pathogen resistance gene (R) or a mutant, variant or derivative
10 thereof, or a pathogen avirulence gene (Avr) or a mutant, variant or derivative thereof, or another R gene elicitor (E), or both (i) an R gene or a mutant, variant, or derivative thereof and (ii) a corresponding Avr gene, or a mutant, variant or derivative thereof,
15 or another R gene elicitor (E).

8. A method according to claim 7 wherein said plant pathogen resistance gene (R) is a tomato Cf-9 gene or a mutant, variant, derivative or homologue thereof and the avirulence gene is a *Cladosporium fulvum* Avr-9 gene
20 or a mutant, variant, derivative or homologue thereof, or encodes another Cf-9 elicitor.

9. A method according to claim 7 wherein said plant pathogen resistance gene (R) is a tomato Cf-2 gene or a mutant, variant, derivative or homologue thereof and
25 the avirulence gene is a *Cladosporium fulvum* Avr-2 gene

or a mutant, variant, derivative or homologue thereof,
or encodes another Cf-2 elicitor; or wherein said plant
pathogene resistance gene (R) is a tomato Cf-4 gene or
a mutant, variant, derivative or homologue thereof and
5 the avirulence gene is a *Cladosporium fulvum* Avr-4 gene
or a mutant, variant, derivative or homologue thereof,
or encodes another Cf-4 elicitor; or wherein said plant
pathogen resistance gene (R) is the tobacco N' gene or
a mutant, variant, derivative or homologue thereof, and
10 the avirulence gene is a suitable Tobacco Mosaic Virus
coat protein, or a mutant, variant, derivative or
homologue thereof or encodes another N' elicitor; or
wherein said plant pathogen resistance gene (R) is the
potato Rx gene or a mutant, variant, derivative or
15 homologue thereof and the avirulence gene is a suitable
PVX coat protein or a mutant, variant, derivative or
homologue thereof or another Rx elicitor; or wherein
said plant pathogen resistance gene is another viral
resistance gene and the avirulence gene encodes a
20 corresponding viral coat protein or other elicitor of
the viral resistance gene.

10. A method according to claim 5 wherein said
nucleotide sequence encodes a Cauliflower Mosaic Virus
gene VI protein, a bacterial harpin gene protein, an
25 *Arabidopsis RPP5* gene protein, a ubiquitin conjugating
enzyme, an RNase such as Barnase, a mutant, variant,
derivative or homologue of any of these, or other toxic

polypeptide or peptide such as diphtheria toxin or a mutant, variant, derivative or homologue thereof.

11. A method according to claim 4 in which the plant defence response or necrosis is dependent on the
5 expression from a nucleotide sequence leading to the reduction of expression of a gene that negatively regulates the plant defence response, resulting in the plant defence response and/or necrosis.

12. A method according to claim 4 in which the plant
10 defence response or necrosis is dependent on the expression of an allele of a gene from a nucleotide sequence which activates the plant defence response in the absence of a ligand that is capable of interacting with the product of said gene, resulting in the plant
15 defence response and/or necrosis.

13. A method according to claim 5 in which the plant defence response or necrosis is dependent on the expression of a mutant allele of a gene from a nucleotide sequence which is capable of activating the
20 plant defence response and the expression of an enfeebled negative regulator of the defence response, leading to the plant defence response and/or necrosis.

14. A method according to any of the preceding claims wherein the inactivation of said nucleotide sequence or

of one or more of said nucleotide sequences is effected by the insertion therein of a transposable genetic element.

15. A method according to claim 14 wherein said
5 transposable genetic element is a transposon or a nucleotide sequence bordered by specific nucleotide sequences that can be recognised by a site specific recombination system.

16. A method according to any of the preceding claims
10 wherein said plant genome comprises at least one nucleotide sequence encoding a substance capable of restoring said inactivated nucleotide sequence or sequences to a functional form to result in increased pathogen resistance.

15 17. A method according to claim 16 which comprises restoring said inactivated nucleotide sequence or sequences to a functional form by excision or rearrangement of said transposable genetic element.

18. A method according to claim 17 wherein when said
20 transposable element is a transposon, said plant genome comprises at least one nucleotide sequence coding for a corresponding transposon activation system to effect somatic excision of said transposon.

19. A method according to claim 18 wherein the genes encoding the transposon and transposase are derived from the Activator/Dissociation transposable element family (Ac/Ds) or from the Enhancer/Suppressor mutator
5 transposon family (En/Spm).

20. A method according to claim 17 wherein when said inactive form of said nucleotide sequence or sequences is flanked by recombinase recognition sequences, said recombinase recognition sequences are acted on by a
10 site specific recombination system which comprises a specific recombinase to result in recombination.

21. A transgenic plant, or descendant thereof, or part or propagule of the plant or descendant, obtainable using a method of any of the preceding
15 claims with increased pathogen resistance compared with wild-type.

22. A plant, or a descendant thereof, or a part or propagule of the plant or descendant, or a derivative of any of these, which is phenotypically variegated,
20 comprising a cell or clone expressing a first phenotype and other cells expressing a second phenotype comprising increased pathogen resistance compared with wild-type.

23. A plant, descendant, derivative, part or

propagule according to claim 22 wherein the first phenotype is necrosis and/or a plant defence response phenotype.

24. A plant, descendant, derivative, part or
5 propagule according to claim 22 or claim 23 wherein the phenotypic variegation results from expression in cells with the first phenotype from a nucleotide sequence or sequences which contribute to such phenotype, said expression from said nucleotide sequence or sequences
10 being inactivated in cells not having said first phenotype.

25. A plant, descendant, derivative, part or
propagule according to claim 24 wherein said expression results from reactivation of a previously inactivated
15 gene.

26. A plant, descendant, derivative, part or
propagule according to claim 24 or claim 25 wherein said inactivation results from insertion of a transposable genetic element into said nucleotide
20 sequence or one or more of said nucleotide sequences.

27. A plant, descendant, derivative, part or
propagule according to any one of claims 24 to 26, wherein said nucleotide sequence or sequences comprises: a gene (R) which is a plant pathogen

resistance gene or a mutant, variant or derivative thereof; or a gene (L) which is a pathogen avirulence gene (Avr) or a mutant, variant or derivative thereof, or another elicitor or ligand gene the product of which
5 can interact with the product of a R-gene; or both an R gene and an L gene.

28. A plant, descendant, derivative, part or propagule according to claim 27 wherein the R gene is a tomato *Cf-9* gene or a mutant, variant, derivative or
10 homologue thereof and the L gene is a *Cladosporium fulvum* *Avr-9* gene or a mutant, variant, derivative or homologue thereof, or encodes another *Cf-9* elicitor.

29. A plant, descendant, derivative, part or propagule according to claim 27 wherein said R gene is:
15 (i) a pathogen resistance gene from tomato;
(ii) a pathogen resistance gene from tobacco;
(iii) a pathogen resistance gene from potato;
(iv) a pathogen resistance gene from *Arabidopsis*;
(v) a pathogen resistance gene from flax;
20 (vi) a nucleotide sequence encoding a CaMV gene VI protein;
(vii) a nucleotide sequence encoding a bacterial harpin gene protein;
(viii) a nucleotide sequence encoding a ubiquitin
25 conjugating enzyme;
(ix) a nucleotide sequence encoding an RNase;

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- (x) a nucleotide sequence encoding a toxic peptide;
- (xi) a mutant, variant, derivative or homologue of any of (i) to (x);

30. A plant, descendant, derivative, part or
5 propagule according to claim 29 wherein said pathogen resistance gene from tomato is selected from *Cladosporium fulvum* resistance genes including Cf-2, Cf-4, Cf-5 and Cf-9; said pathogen resistance gene from tobacco is N'; said pathogen resistance gene from
10 potato is Nx; said pathogen resistance gene from *Arabidopsis* is RPP5 or RP52; said pathogen resistance gene from flax is L6; said RNase is Barnase; or said toxic peptide is diphtheria toxin.

31. A plant, descendant, derivative, part or
15 propagule according to claim 27 wherein said L gene is:
(i) a *Cladosporium fulvum* avirulence gene or another elicitor of a resistance gene for a *Cladosporium fulvum* avirulence gene;
(ii) a suitable TMV coat protein or another N'
20 elicitor;
(iii) a suitable PVX coat protein or another Rx elicitor; or
(iv) a mutant, variant, derivative or homologue of any of (i) to (iii).

25 32. A plant, descendant, derivative, part or

propagule according to claim 31 wherein said
Cladosporium fulvum avirulence gene is Avr2, Avr4, Avr5
or Avr9.

33. A cell containing (i) nucleic acid encoding one
5 or more than one nucleotide sequence which causes or
contributes to the plant defence response and/or cell
necrosis, at least one said nucleotide sequence being
reversibly inactivated and (ii) nucleic acid encoding a
molecule or molecules able to reverse the inactivation.
- 10 34. A cell according to claim 33 wherein the
inactivation results from insertion of a transposable
genetic element into one or more of said nucleotide
sequences.
- 15 35. A cell according to claim 34 wherein said
transposable genetic element is a transposon and said
molecule or molecules provide a corresponding
transposon activation system to effect excision of said
transposon.
- 20 36. A cell according to any one of claims 33 to 35
wherein said nucleotide sequence or sequences
comprises: a gene (R) which is a plant pathogen
resistance gene or a mutant, variant or derivative
thereof; or a gene (L) which is a pathogen avirulence
gene (Avr) or a mutant, variant or derivative thereof,

or another elicitor or ligand gene the product of which can interact with the product of a R-gene; or both an R gene and an L gene.

37. A cell according to claim 36 wherein the R gene
5 is a tomato *Cf-9* gene or a mutant, variant, derivative or homologue thereof and the L gene is a *Cladosporium fulvum* *Avr-9* gene or a mutant, variant, derivative or homologue thereof, or encodes another *Cf-9* elicitor.

38. A cell according to claim 37 wherein said R gene
10 is:

- (i) a pathogen resistance gene from tomato;
- (ii) a pathogen resistance gene from tobacco;
- (iii) a pathogen resistance gene from potato;
- (iv) a pathogen resistance gene from *Arabidopsis*;
- 15 (v) a pathogen resistance gene from flax;
- (vi) a nucleotide sequence encoding a CaMV gene VI protein;
- (vii) a nucleotide sequence encoding a bacterial harpin gene protein;
- 20 (viii) a nucleotide sequence encoding a ubiquitin conjugating enzyme;
- (ix) a nucleotide sequence encoding an RNase;
- (x) a nucleotide sequence encoding a toxic peptide;
- (xi) a mutant, variant, derivative or homologue of
- 25 any of (i) to (x);

39. A cell according to claim 38 wherein said pathogen resistance gene from tomato is selected from *Cladosporium fulvum* resistance genes including Cf-2, Cf-4, Cf-5 and Cf-9; said pathogen resistance gene from tobacco is N'; said pathogen resistance gene from potato is Nx; said pathogen resistance gene from *Arabidopsis* is RPP5 or RP52; said pathogen resistance gene from flax is L6; said RNase is Barnase; or said toxic peptide is diphtheria toxin.
40. A cell according to claim 36 wherein said L gene is:
- (i) a *Cladosporium fulvum* avirulence gene or another elicitor of a resistance gene for a *Cladosporium fulvum* avirulence gene;
 - (ii) a suitable TMV coat protein or another N' elicitor;
 - (iii) a suitable PVX coat protein or another Rx elicitor; or
 - (iv) a mutant, variant, derivative or homologue of any of (i) to (iii).
41. A cell according to claim 40 wherein said *Cladosporium fulvum* avirulence gene is Avr2, Avr4, Avr5 or Avr9.
42. A cell according to any one of claims 33 to 41 which is a microbial cell.

43. A cell according to any one of claims 33 to 41 which is a plant cell.

44. A plant or any part or propagule or derivative thereof comprising a cell according to claim 43.

5 45. A plant, part, propagule or derivative according to claim 44 which is variegated for cells wherein said nucleotide sequence is inactivated or activated.

46. A method of producing a cell according to any one of claims 33 to 45 comprising introduction of nucleic
10 acid (i) and/or (ii) into the cell or an ancestor thereof.

47. A composition of matter comprising any of the following combinations of nucleotide sequences:

(i) a nucleotide sequence comprising R, a nucleotide
15 sequence comprising I and a nucleotide sequence comprising A;

(ii) a nucleotide sequence comprising R, and a nucleotide sequence comprising I and A;

(iii) a nucleotide sequence comprising I, and a
20 nucleotide sequence comprising A and R;

(iv) a nucleotide sequence comprising A, and a nucleotide sequence comprising R and I; and

(v) a nucleotide sequence comprising R, I and A;

wherein R encodes a substance whose presence in a plant

results in a plant defence response, necrosis and/or increased pathogen resistance, I is a genetic insert able to inactivate R and A encodes a substance able to reactivate R inactivated by I.

- 5 48. A composition of matter comprising any of the following combinations of nucleotide sequences:
- (i) a nucleotide sequence comprising R, a nucleotide sequence comprising L, a nucleotide sequence comprising I, and a nucleotide sequence comprising A;
 - 10 (ii) a nucleotide sequence comprising R, a nucleotide sequence comprising L and I, and a nucleotide sequence comprising (A);
 - (iii) a nucleotide sequence comprising R, a nucleotide sequence comprising L and A, and a nucleotide sequence
 - 15 comprising I;
 - (iv) a nucleotide sequence comprising R, a nucleotide sequence comprising I and A, and a nucleotide sequence comprising L;
 - (v) a nucleotide sequence comprising L, a nucleotide
 - 20 sequence comprising I and R, and a nucleotide sequence comprising A;
 - (vi) a nucleotide sequence comprising L, a nucleotide sequence comprising A and R, and a nucleotide sequence comprising I;
 - 25 (vii) a nucleotide sequence comprising I, a nucleotide sequence comprising L and R, and a nucleotide sequence comprising A;

- (viii) a nucleotide sequence comprising R, and a nucleotide sequence comprising L, I and A;
- (ix) a nucleotide sequence comprising L, and a nucleotide sequence comprising I, A and R;
- 5 (x) a nucleotide sequence comprising I, and a nucleotide sequence comprising A, R and L;
- (xi) a nucleotide sequence comprising A and a nucleotide sequence comprising A, R and I; and
- (xii) a nucleotide sequence comprising R, L, I and A;
- 10 wherein R and L encode substances whose presence together in a plant results in a plant defence response, necrosis and/or increased pathogen resistance, I is a genetic insert able to inactivate R and/or L and A encodes a substance able to reactivate R
- 15 and/or L inactivated by I.

49. A composition of matter according to claim 47 or 48 which is one or more nucleic acid vectors.

50. A composition of matter according to any one of claims 47 to 49 wherein a cell contains any of said

20 combinations of nucleotide sequences.

51. A plant, or a part, propagule, derivative or descendant thereof, comprising a cell according to the composition of claim 50.

52. A method of producing a plant, or a part,

propagule, derivative or descendant thereof, containing nucleic acid comprising a nucleotide sequence or nucleotide sequences encoding R, I and A, wherein R encodes a substance whose presence in a plant results in a plant defence response, necrosis and/or increased pathogen resistance, I is a genetic insert able to inactivate R and A encodes a substance able to reactivate R inactivated by I, comprising crossing plant lines whose genomes comprise any of R, I, A and combinations thereof, to produce the plant or an ancestor thereof.

53, A method according to claim 52 wherein one or more of said plant lines contains nucleic acid comprising any of R, I, A and combinations thereof as a result of transformation of cells of the plant or an ancestor thereof.

54. A method of producing a plant, or a part, propagule, derivative or descendant thereof, containing nucleic acid comprising a nucleotide sequence or nucleotide sequences encoding R, L, I and A, wherein R and L encode substances whose presence together in a plant results in a plant defence response, necrosis and/or increased pathogen resistance, I is a genetic insert able to inactivate R and/or L and A encodes a substance able to reactivate R and/or L inactivated by I, comprising crossing plant lines whose genomes

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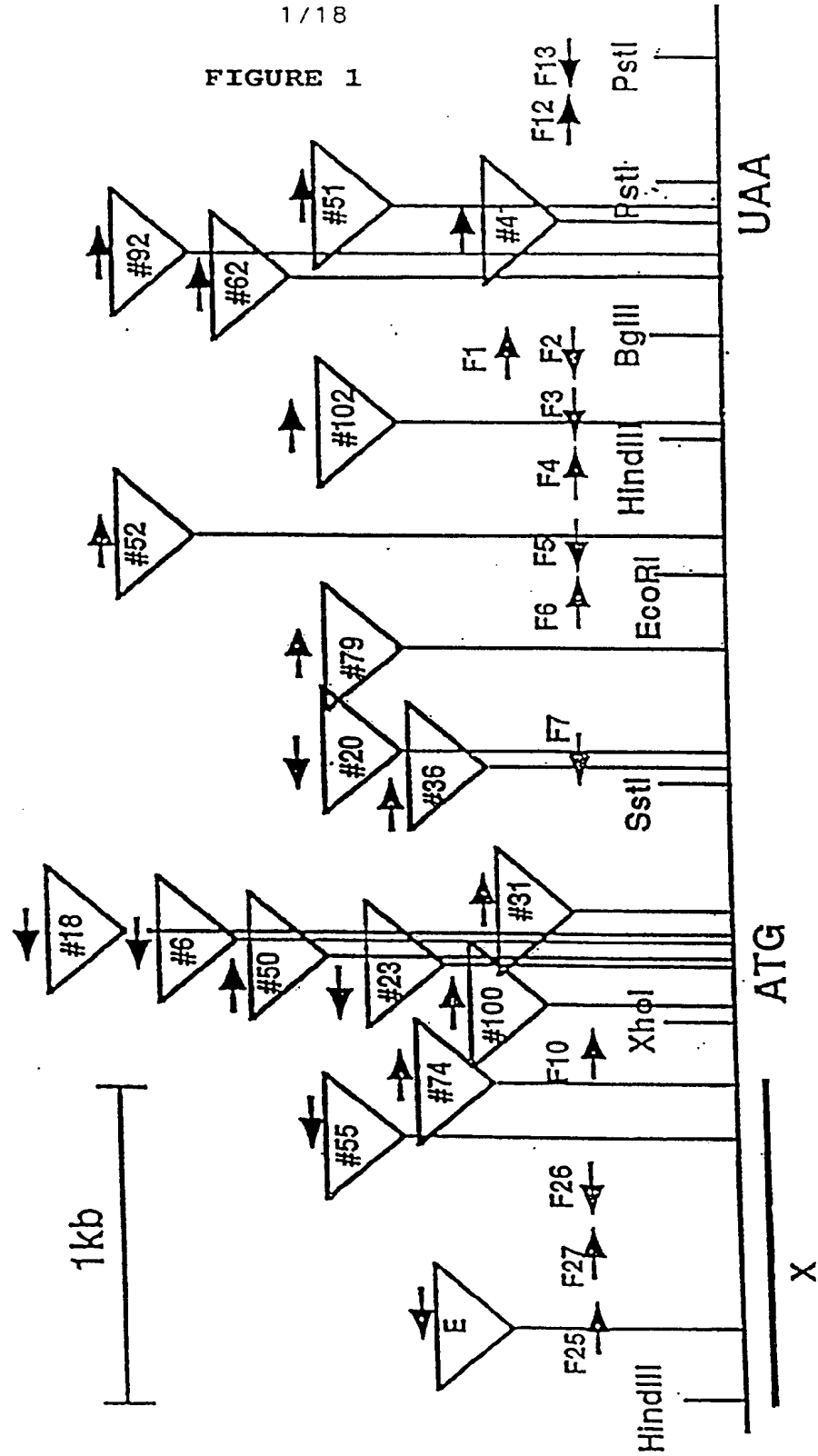
comprise any of R, L, I, A and combinations thereof, to produce the plant or an ancestor thereof.

55, A method according to claim 54 wherein one or more of said plant lines contains nucleic acid
5 comprising any of R, L, I, A and combinations thereof as a result of transformation of cells of the plant or an ancestor thereof.

56. A plant, or a part, propagule, derivative or descendant thereof, obtainable using a method according
10 to any one of claims 52 to 55.

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FIGURE 1



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FIGURE 2

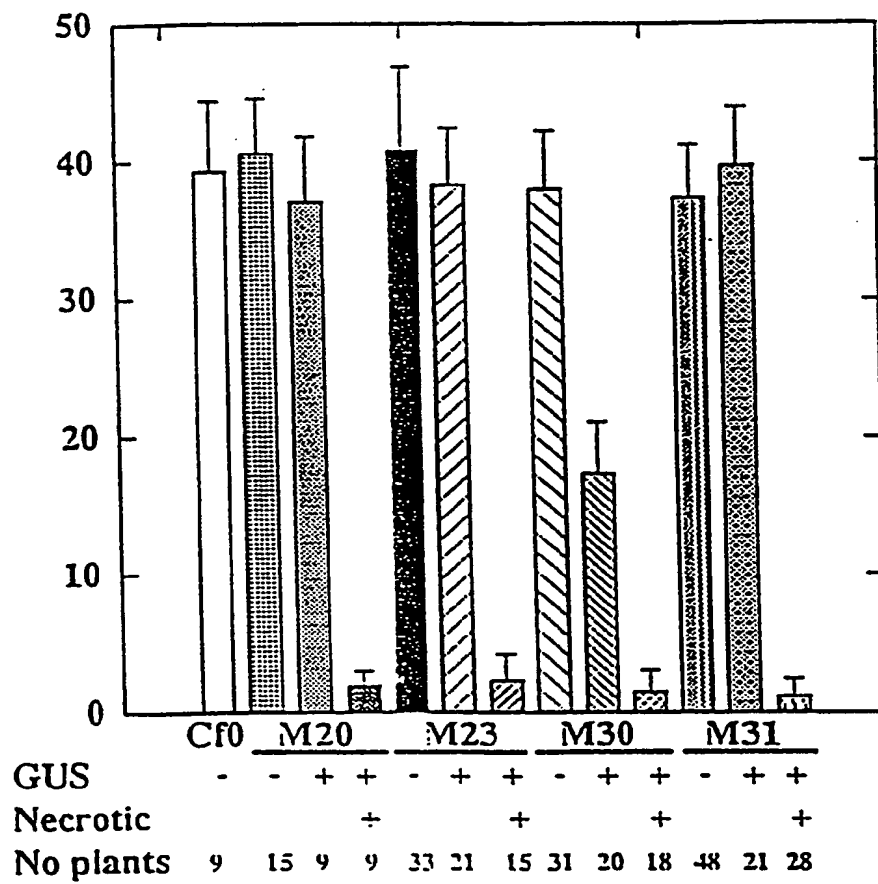
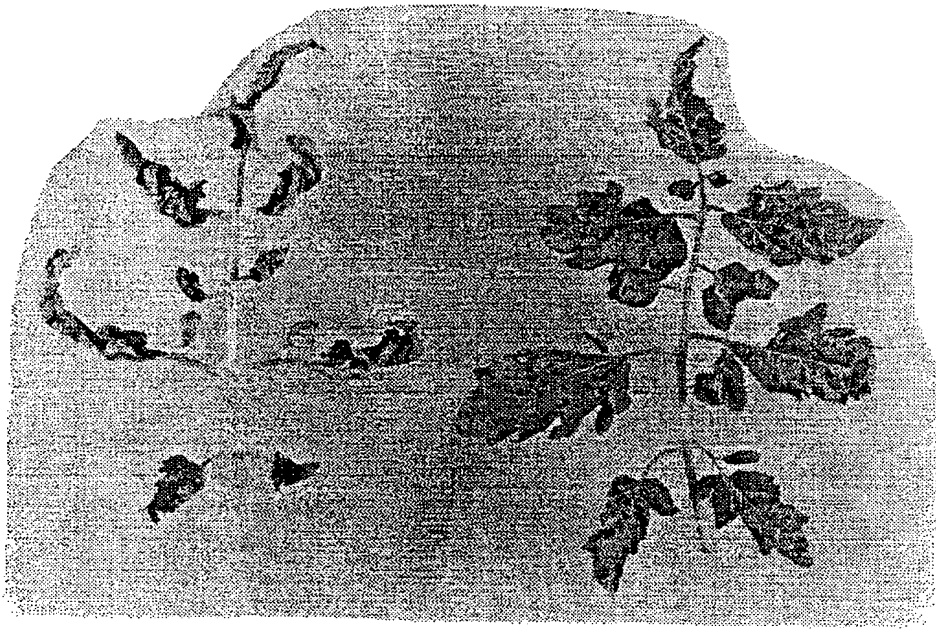


FIGURE 3A



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FIGURE 3B

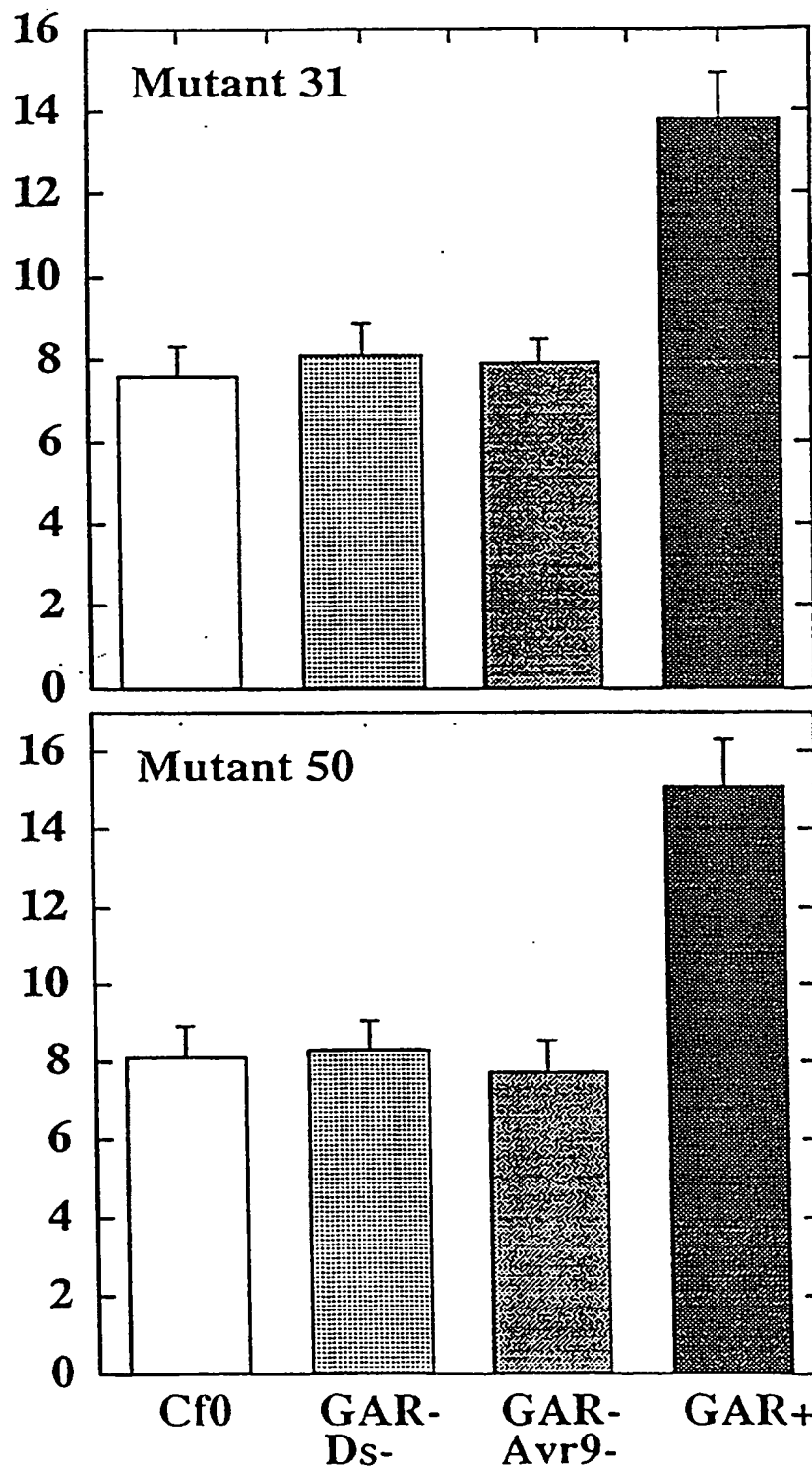
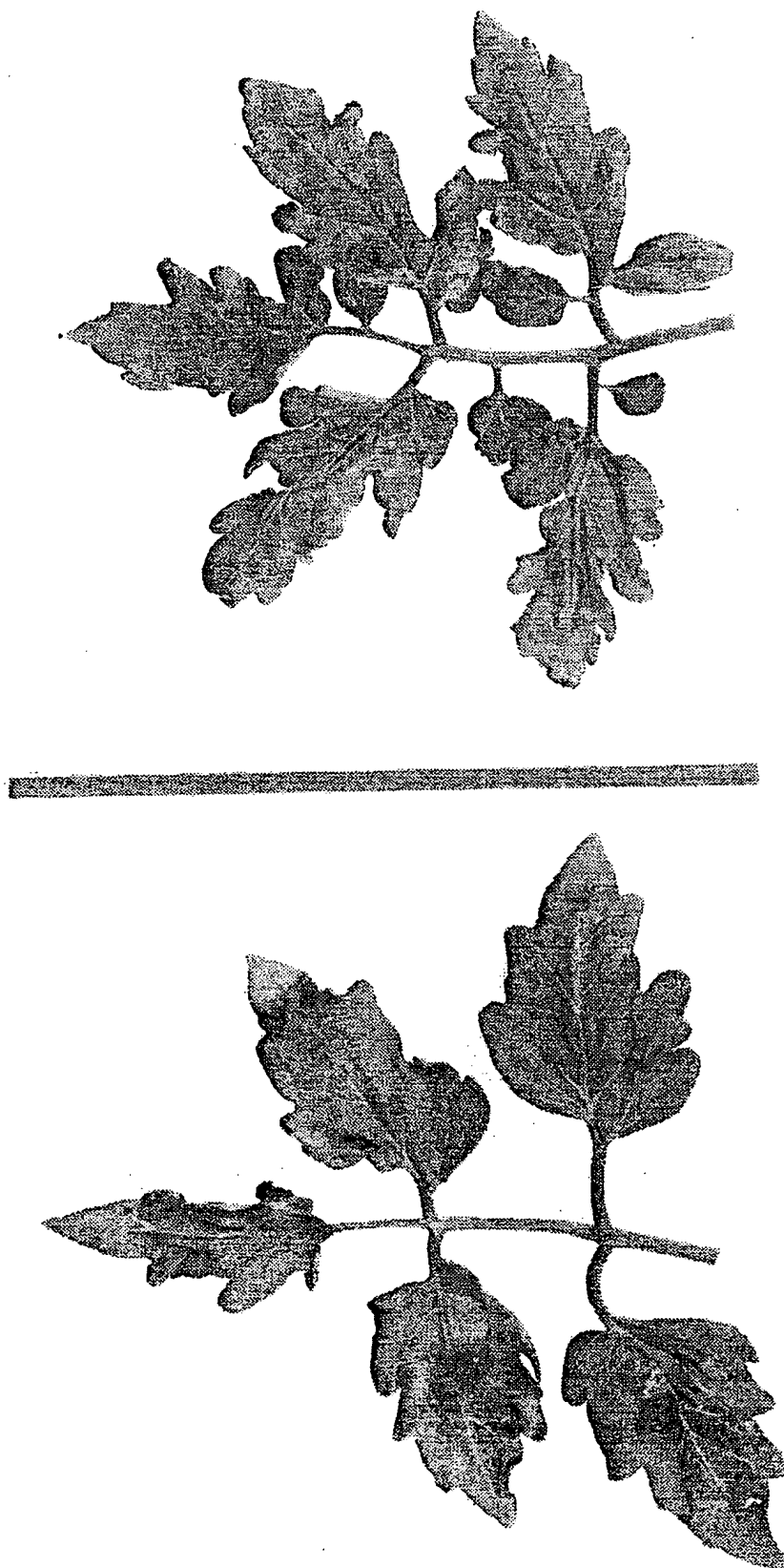
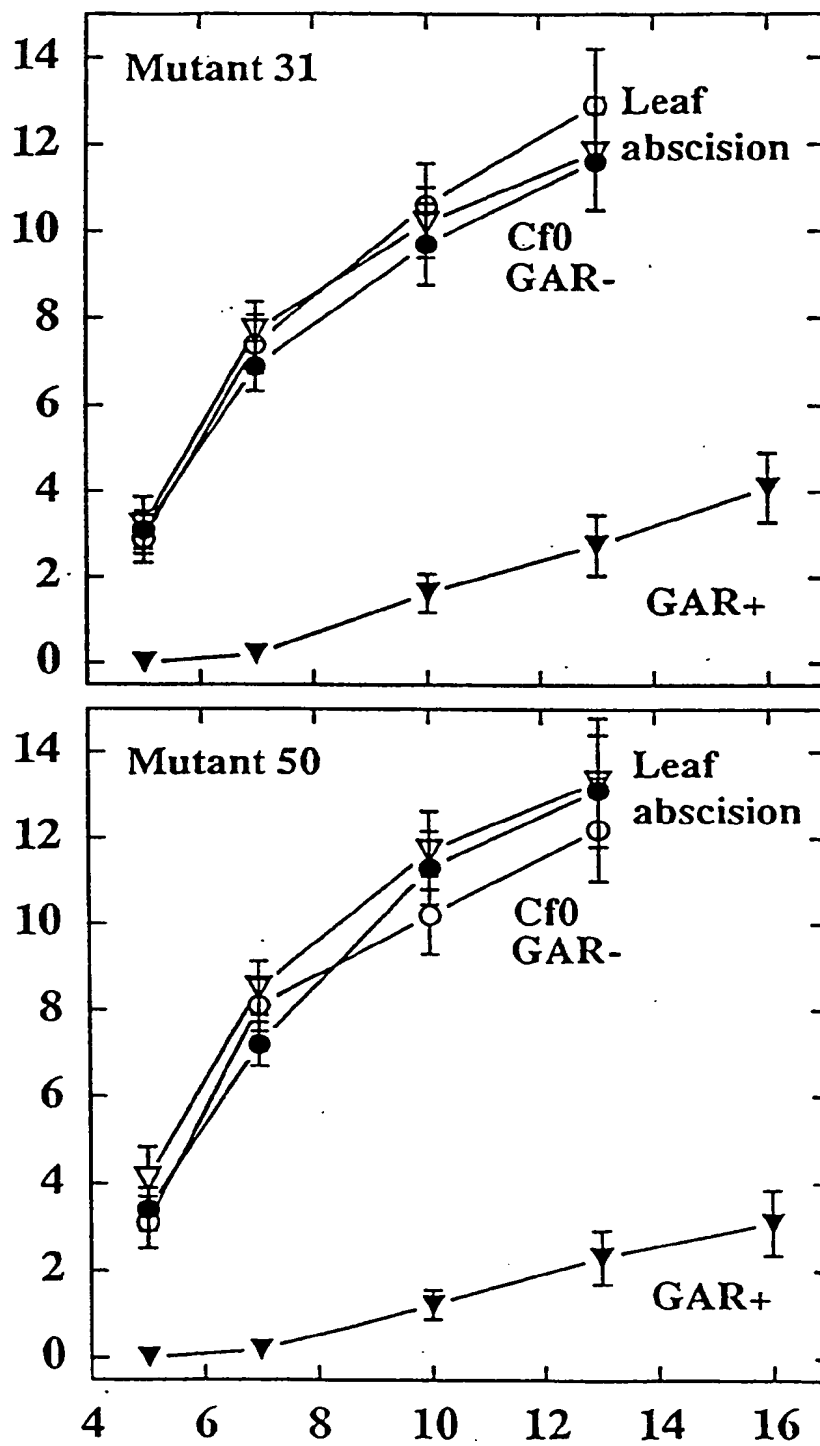


FIGURE 4A



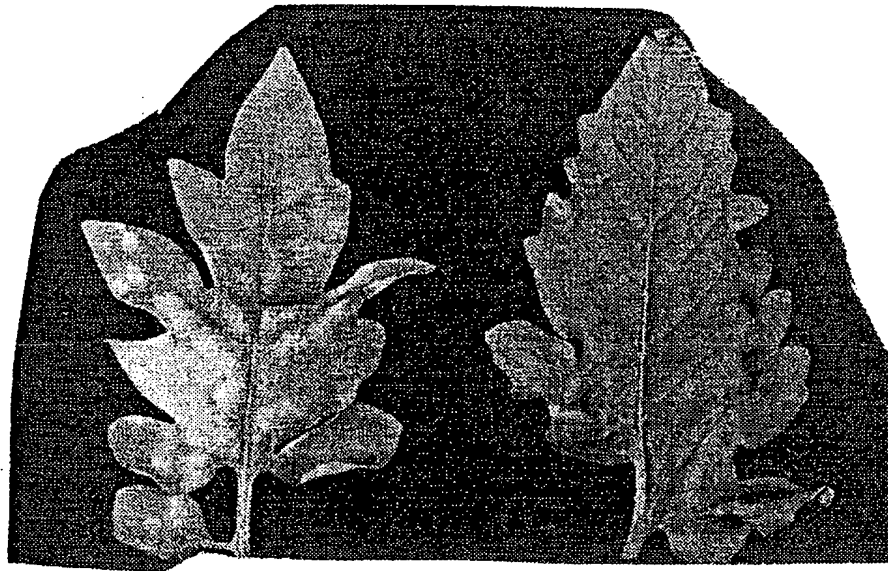
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FIGURE 4B



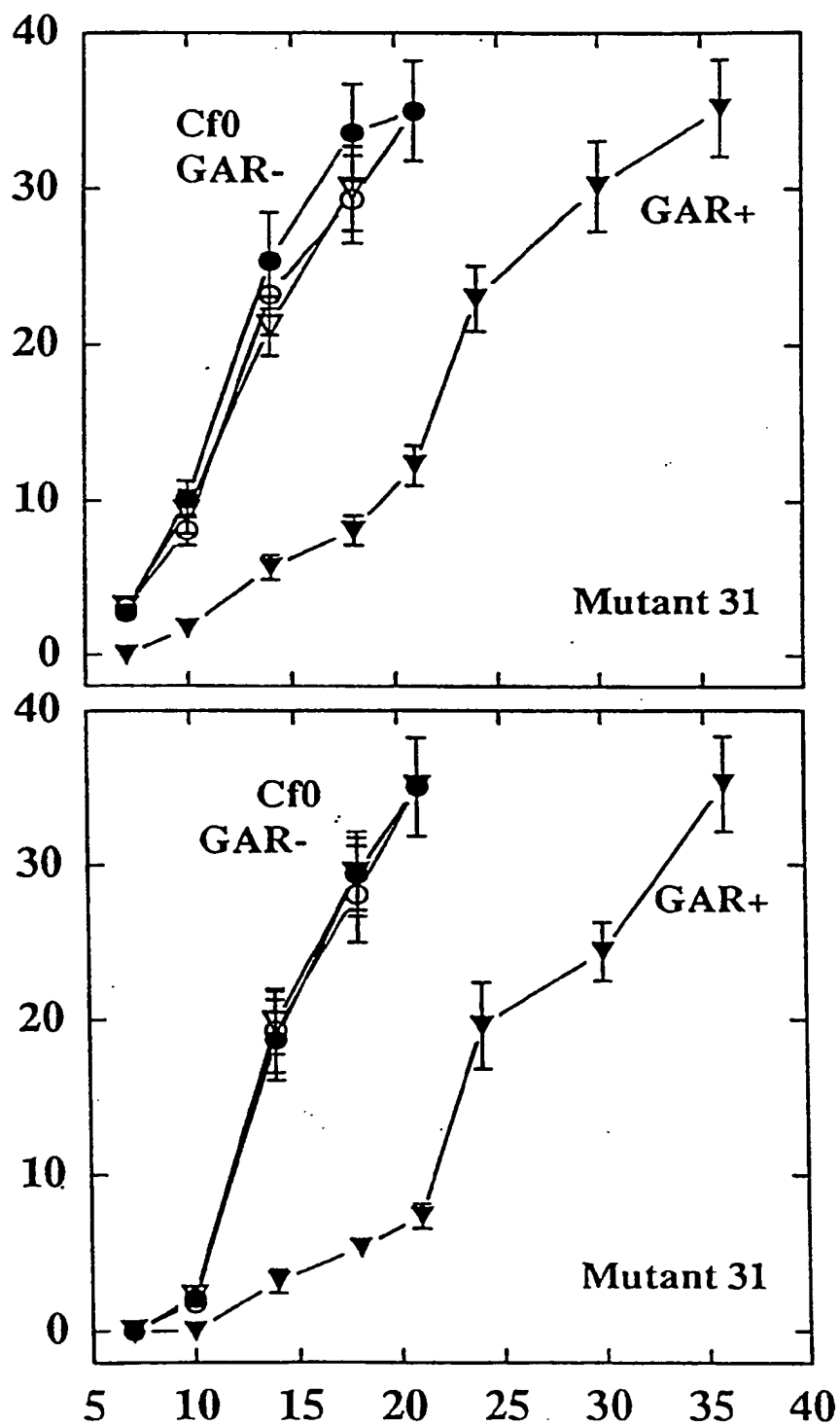
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FIGURE 5



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FIGURE 5B



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FIGURE 5C

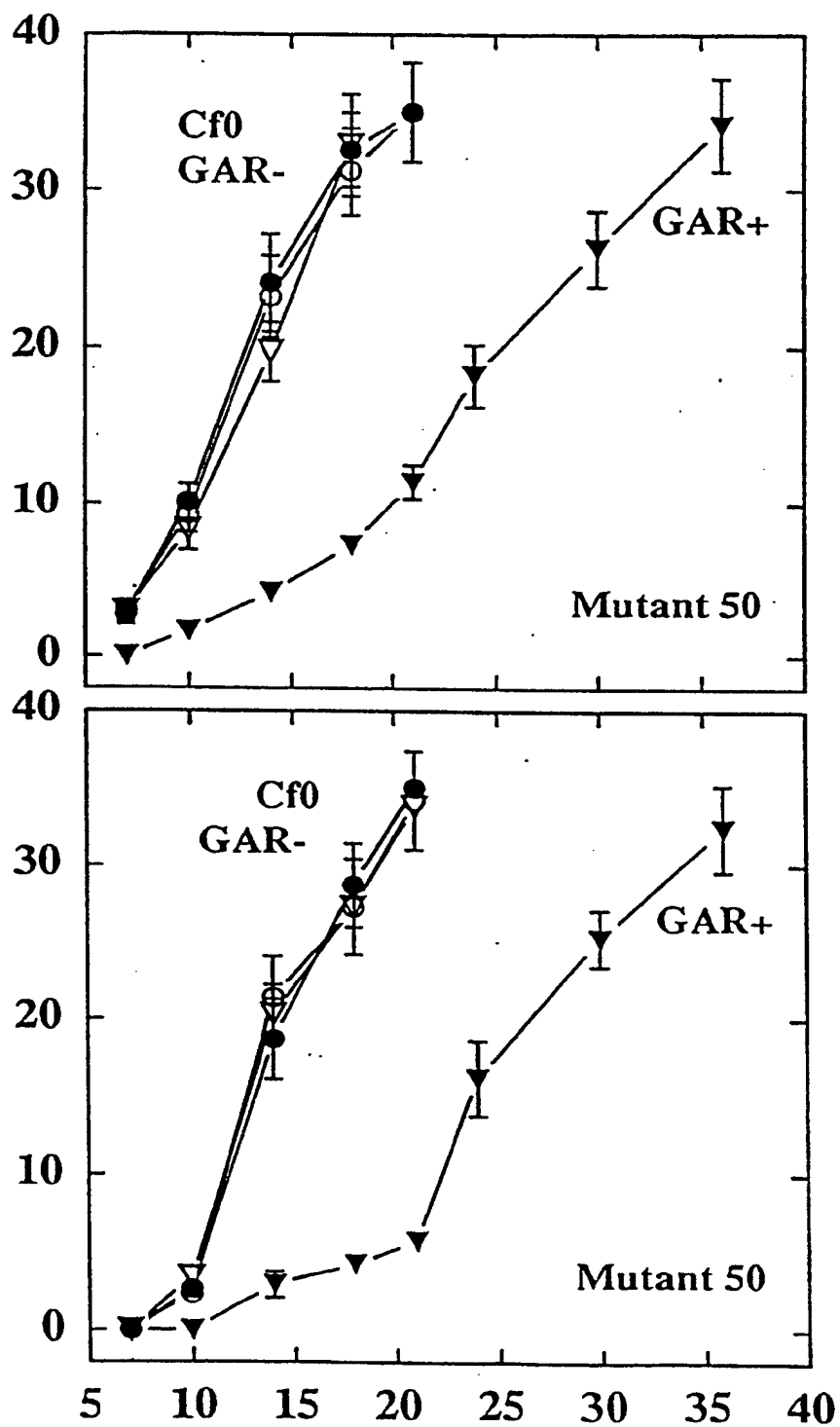
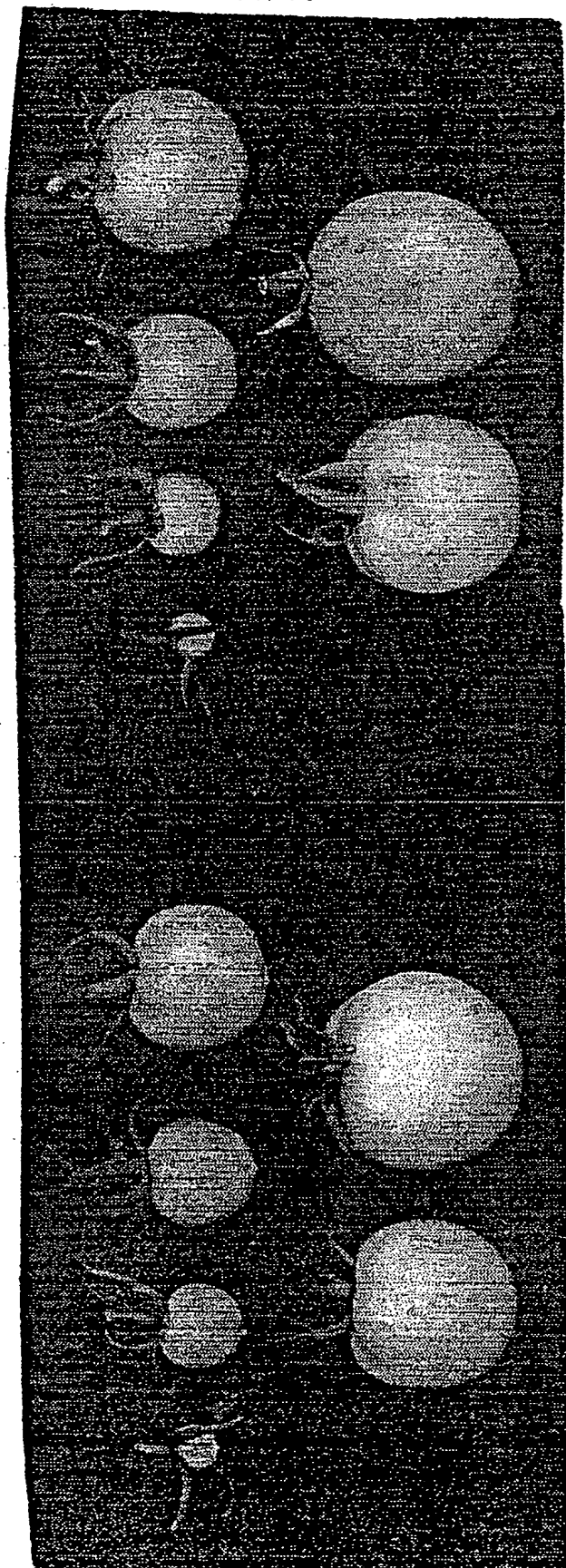
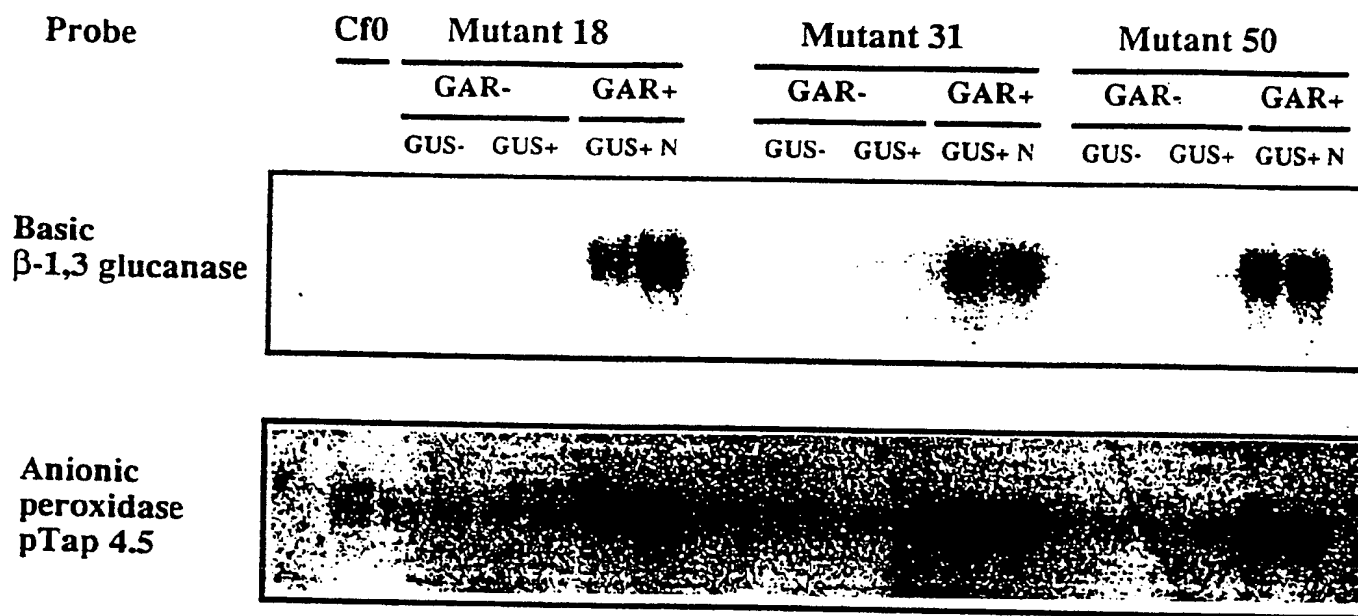


FIGURE 6



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FIGURE 7



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FIGURE 8B

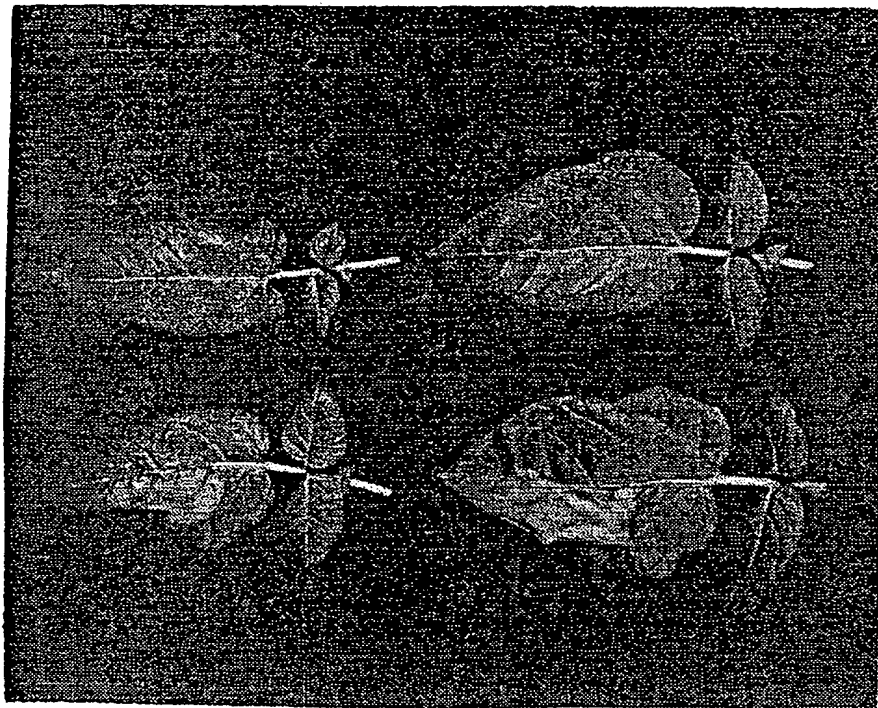


FIGURE 8A

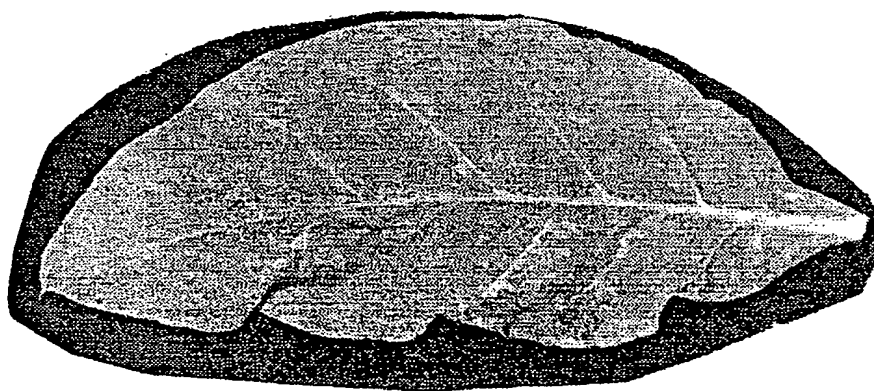
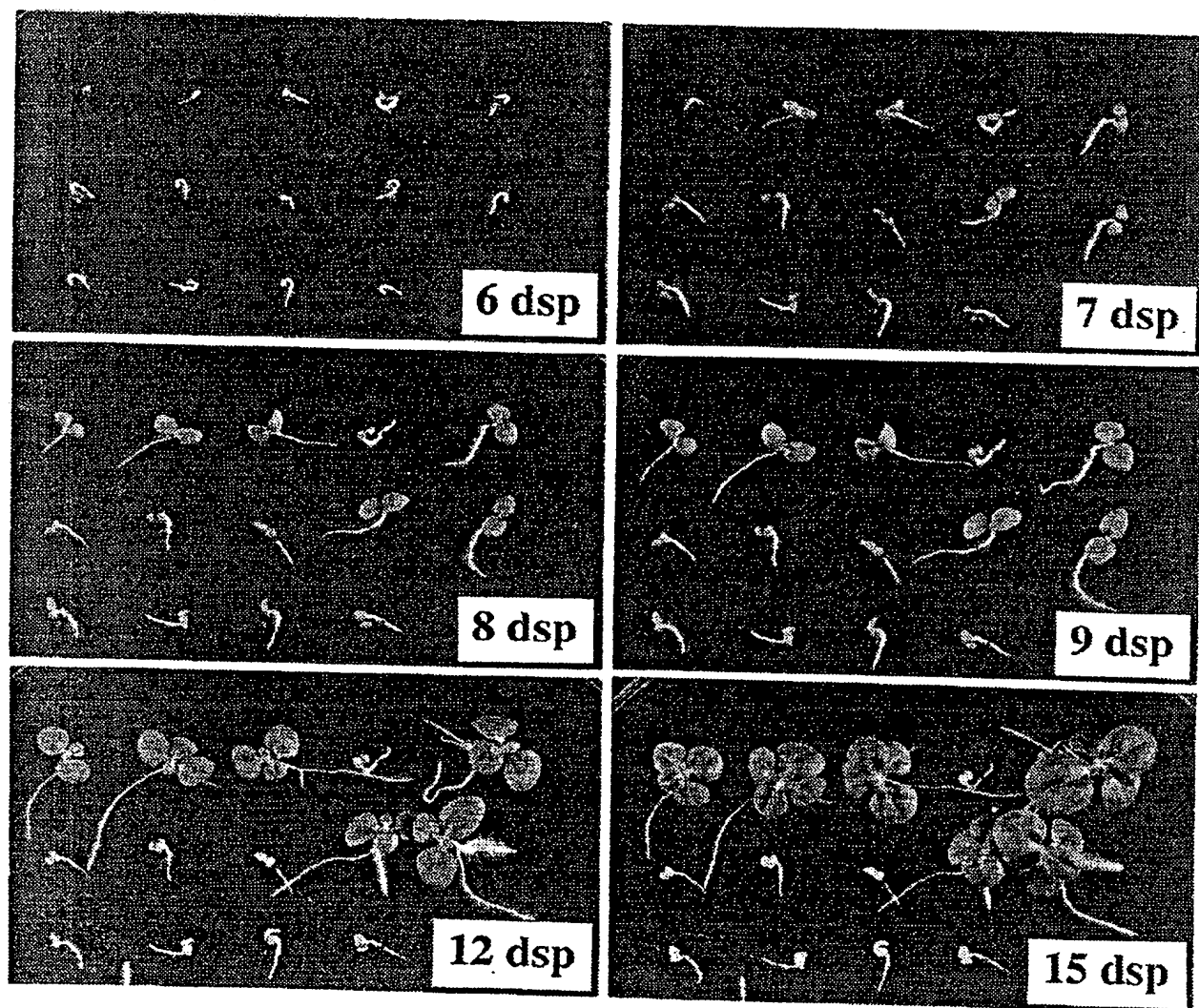


FIGURE 9



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FIGURE 10

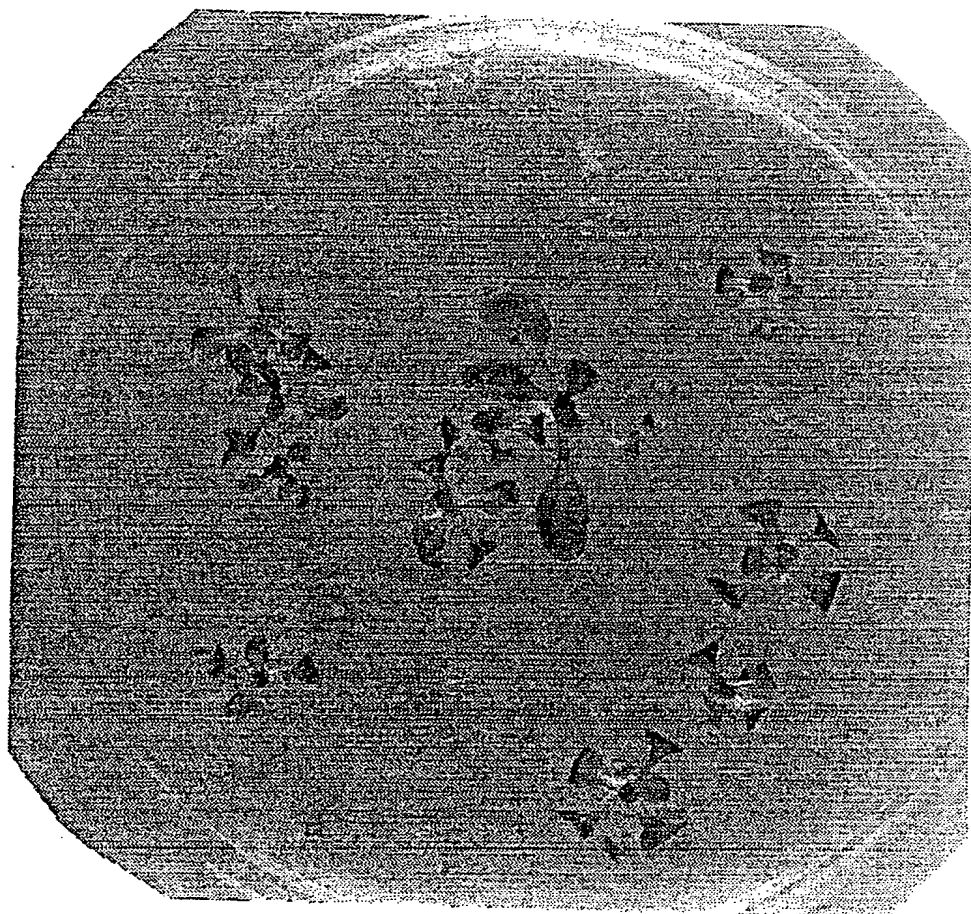


FIGURE 11

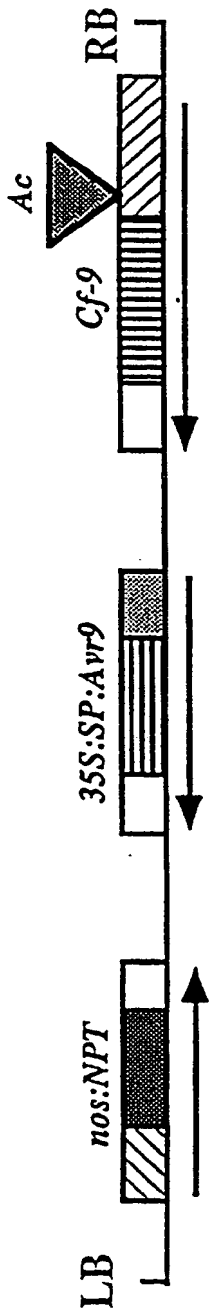
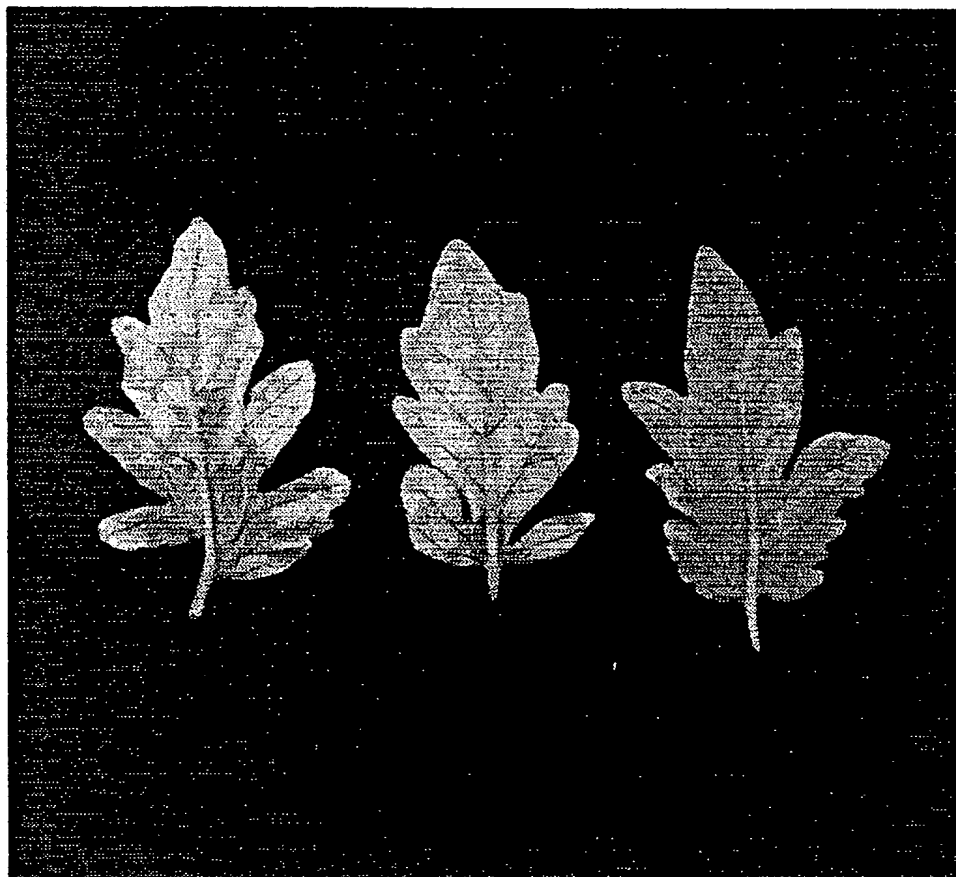


Figure 12



Leaf 1

Leaf 2

Leaf 3

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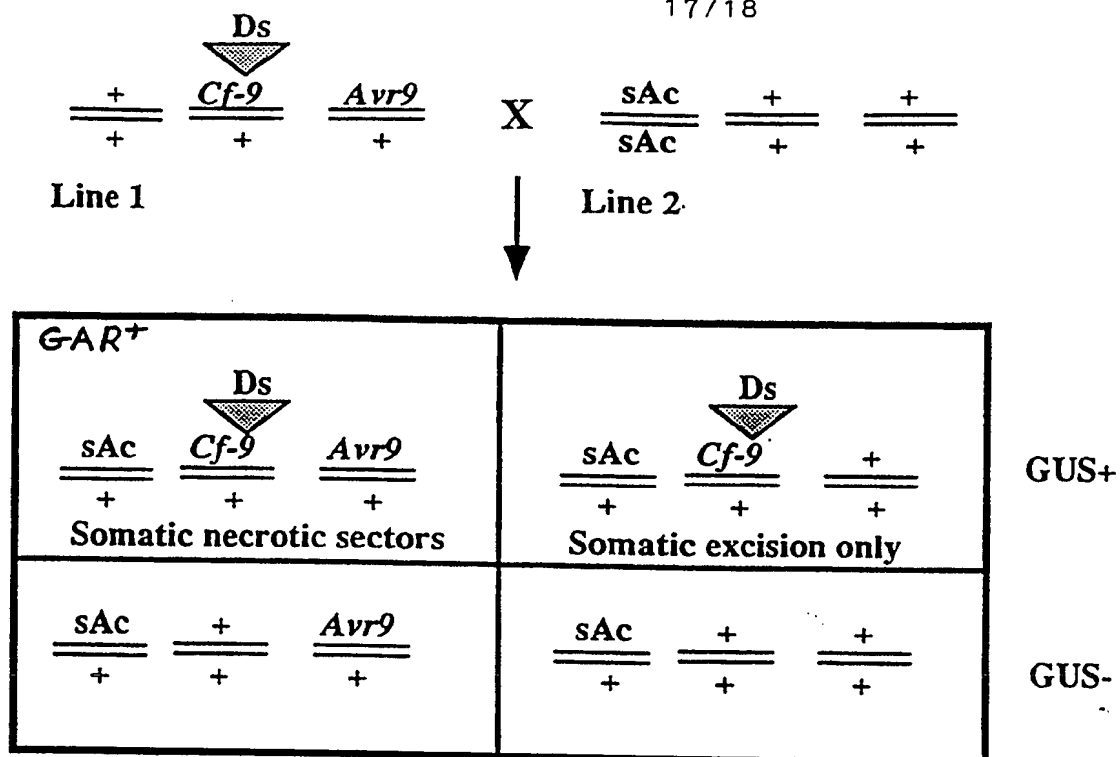
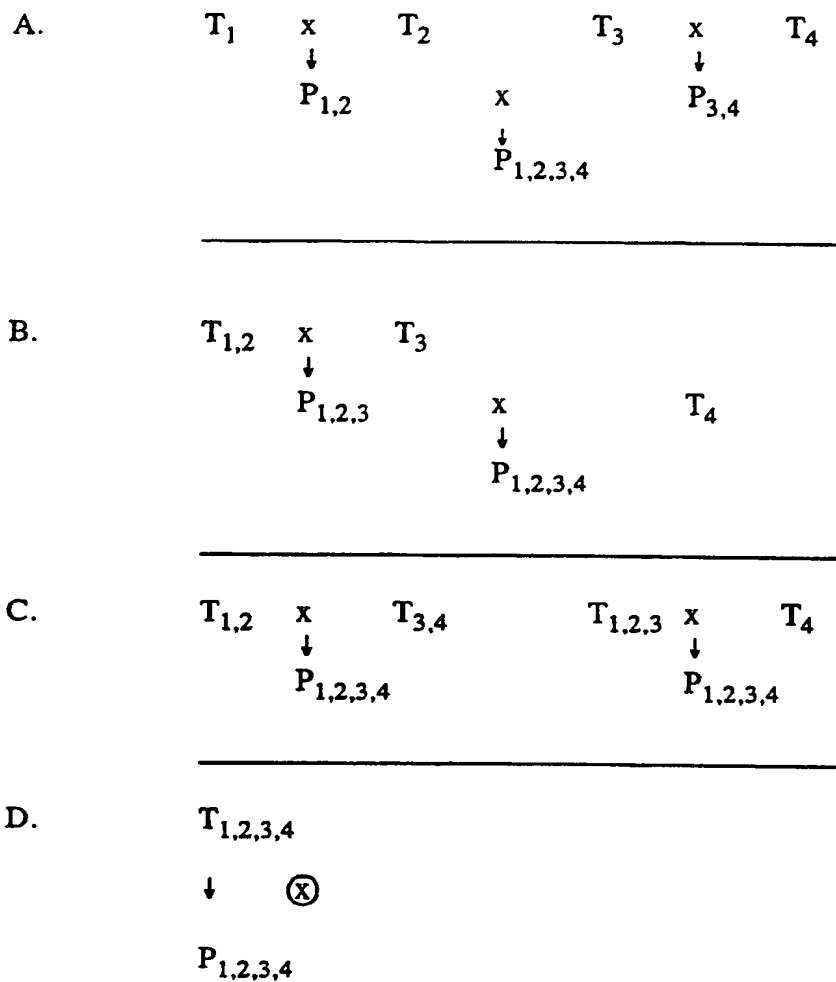


FIGURE 13

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FIGURE 14



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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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			(43) International Publication Date: 23 November 1995 (23.11.95)
(21) International Application Number: PCT/GB95/01075		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).	
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(30) Priority Data: 9409394.5 11 May 1994 (11.05.94) GB PCT/GB94/02812 24 December 1994 (24.12.94) WO (34) Countries for which the regional or international application was filed: GB et al. 9506658.5 31 March 1995 (31.03.95) GB 9507232.8 7 April 1995 (07.04.95) GB			
(71) Applicant (for all designated States except US): THE GATSBY CHARITABLE FOUNDATION [GB/GB]; 9 Red Lion Court, London EC4A 3EB (GB).		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(72) Inventors; and (75) Inventors/Applicants (for US only): JONES, Jonathan, Dallas, George [GB/GB]; 19 Waverley Road, Norwich, Norfolk NR4 6SG (GB). HAMMOND-KOSACK, Kim, Elizabeth [GB/GB]; 6 Chestnut Hill, Norwich RN4 6NL (GB). JONES, David, Allen [GB/GB]; 139 Greenways, Eaton, Norwich NR4 6PD (GB).		(88) Date of publication of the international search report: 14 December 1995 (14.12.95)	
(74) Agents: WALTON, Seán, M. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).			
(54) Title: METHOD OF INTRODUCING PATHOGEN RESISTANCE IN PLANTS			
(57) Abstract			
<p>Variegated plants have increased pathogen resistance: cells of the plant express a phenotype, which may comprise necrosis and/or a plant defence response, and other cells not expressing this phenotype have increased pathogen resistance. Embodiments of the invention employ various genes, including <i>Cladosporium fulvum</i> pathogen resistance genes, which are inactivated, for example as a result of insertion of a transposable genetic element, and then reactivated in plant cells to result in necrosis and/or a plant defence response, leading to increased pathogen resistance. Cells, plants and other compositions of matter are provided comprising various combinations of genes involved in this system.</p>			

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INTERNATIONAL SEARCH REPORT

 Internatio Application No
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 A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/82 C12N15/29 C12N15/31 C12N5/10 A01N63/02
 A01N65/00 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO,A,92 13090 (GEN HOSPITAL CORP) 6 August 1992 see page 11, line 21 - page 12, line 2 see page 33, line 13 - line 20; example 2 ---	1-4,21, 33,43-45
X	WO,A,92 13089 (GEN HOSPITAL CORP ; HARVARD COLLEGE (US)) 6 August 1992 see page 12, line 3 - line 24 see page 35, line 3 - line 10; example 2 ---	1-4,21, 33,43-45
A	WO,A,91 15585 (RIJKSLANDBOUWHOGESCHOOL) 17 October 1991 see the whole document ---	1-56

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Date of the actual completion of the international search

19 October 1995

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A	<p>SCIENCE, vol. 262, November 1993 LANCASTER, PA US, pages 1432-1436, MARTIN, G.B., ET AL. 'MAP-BASED CLONING OF A PROTEIN KINASE GENE CONFERRING DISEASE RESISTANCE IN TOMATO' see the whole document ---</p>	1-56
E	<p>WO,A,95 18230 (GATSBY CHARITABLE FOUNDATION ;JONES JONATHAN DALLAS GEORGE (GB); H) 6 July 1995 see page 25, line 1 - line 8 -----</p>	21-56

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatio Application No

PCT/GB 95/01075

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